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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 48/00, C12N 5/00, 15/00	A1	(11) International Publication Number: WO 97/00698 (43) International Publication Date: 9 January 1997 (09.01.97)

(21) International Application Number: PCT/US96/10602

(22) International Filing Date: 20 June 1996 (20.06.96)

(30) Priority Data:
60/017,814 20 June 1995 (20.06.95) US

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(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: INHIBITION OF HEPATITIS B REPLICATION

(57) Abstract

The invention features a method of inhibiting the replication of a naturally-occurring hepadnavirus, e.g., hepatitis B virus (HBV), by introducing into proximity with the hepadnavirus a nucleic acid that encodes a hepadnavirus mutant polypeptide. The polypeptide includes a first amino acid sequence that is substantially identical to a corresponding region of a wild type hepadnavirus core protein, and either lacks a second amino acid sequence of the wild type hepadnavirus core protein, the second sequence including the carboxyterminal three amino acids of the wild type hepadnavirus core protein, and/or is joined by a peptide bond to the aminoterminal amino acid of an amino acid sequence that is substantially identical to a corresponding portion of a wild type hepadnavirus surface protein, the aminoterminal amino acid of the surface protein being joined by a peptide bond to the carboxyterminal amino acid of the core protein sequence.

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INHIBITION OF HEPATITIS B REPLICATION

The invention was funded in part by grants CA-35711 and AA-08169 from the National Institutes of Health. The Government has certain rights to this invention.

Background of the Invention

The invention relates to treating infections of a hepadnavirus, e.g., hepatitis B virus.

Hepatitis B virus (HBV) is a member of the hepadnavirus family, a group of enveloped DNA viruses that cause acute and chronic hepatitis. Major clinical consequences of HBV infection include acute liver failure, liver cirrhosis, and primary hepatocellular carcinoma (HCC). With more than 250 million individuals infected worldwide, effective treatment of chronic HBV infection is a major public health goal (Ganem et al., *Annu. Rev. Biochem.*, 56: 651-693, 1987). Although an effective and inexpensive vaccine is available for preventing infection, to date there is no effective therapy for treating individuals with persistent infection, nor for reducing the risk of liver disease in infected patients (Maynard et al., *Rev. Infect. Dis.*, 11, S574-S578, 1989); DiBisceglie et al., *Cancer Detection and Prevention*, 14, 291-293, 1989). Current treatments for chronic HBV infection include interferon and other inhibitors of viral DNA synthesis. Since these agents have achieved only limited success, additional antiviral approaches are urgently needed.

Hepadnaviruses are composed of a viral envelope, a nucleocapsid which contains a relaxed circular 3.2 kb DNA genome, and a virally encoded reverse transcriptase. Following infection of a cell, virion DNA is delivered to the nucleus where it is converted into a covalently closed circular DNA (cccDNA), which is in turn

transcribed into several subgenomic and pregenomic mRNAs. The pregenomic RNA is then encapsidated into the viral nucleocapsid, together with the reverse transcriptase enzyme necessary to generate the viral DNA genome (Enders et al., *J. Virol.*, 67, 35-41, 1987). Selective encapsidation of pregenomic RNA depends on both nucleocapsid protein and on viral polymerase (Bartenschlager et al., *J. Virol.*, 64, 5324-5332, 1990; Hirsch et al., *Nature*, 344, 552-555, 1990; Nassal, M., *J. Virol.*, 66, 4107-4116, 1992; Roychoury et al., *J. Virol.*, 65, 3617-3624, 1991) as well as on a cis-acting encapsidation signal located at the 5' end of the pregenomic RNA (Bartenschlager et al., *supra*; Junker-Niepmann et al., *EMBO J.*, 9, 3389-3396, 1990; Pollack et al., *J. Virol.*, 67, 3254-3263, 1993.

The mammalian hepadnavirus 21 kd core protein is a 183-187 (depending on the viral strain) amino acid monomer, 180 of which self assemble into an icosahedral structure within the cytoplasm of infected cells. The core protein has two functional domains. The aminoterminus (amino acids 1 to 139-44) is essential for core assembly. A carboxyterminal arginine-rich region (amino acids 139-183, or 144-187, depending upon the viral strain) binds nucleic acids that are required for positive strand DNA synthesis, and stabilizes core particles for complete assembly of the complex into an enveloped viral particle (Birnbaum et al. *J. Virol.*, 64, 3319-3330, 1990; Yu et al., *J. Virol.*, 65, 2511-2517, 1990; Nassal, M., *supra*).

Summary of the Invention

The invention is based on Applicants' discovery that altering the carboxyterminus of the hepadnavirus core protein creates a mutant polypeptide that reduces replication of a wild type hepadnavirus, by a dominant

negative mechanism. The inhibitory effect is achieved by deletion of a few carboxyterminal amino acids from the core protein, and/or by joining the core protein to a hepadnavirus surface protein, thereby creating a core-surface fusion polypeptide.

Accordingly, the invention features a method of inhibiting the replication of a naturally-occurring, infectious hepadnavirus. The method involves introducing into the proximity of the hepadnavirus a hepadnavirus mutant polypeptide, or a nucleic acid that encodes such a hepadnavirus mutant polypeptide. The polypeptide includes a first amino acid sequence that is substantially identical to a region of a wild type hepadnavirus core protein, but lacks a second amino acid sequence of the wild type hepadnavirus core protein, wherein the second sequence includes the carboxyterminal three amino acids of the wild-type hepadnavirus core protein and does not exceed 100 amino acids in length. The mutant polypeptide is introduced into the infected cell, or is expressed from the nucleic acid, in the proximity of the naturally-occurring hepadnavirus, so as to be available to inhibit replication of the hepadnavirus.

When the method of inhibiting hepadnavirus replication is targeted against HBV, the carboxyterminal amino acid of the first amino acid sequence can be selected from the group consisting of any of the amino acids between position 81 and position 180 of the sequence shown in Fig. 15 (SEQ ID NO: 12), inclusive; preferably the carboxyterminal amino acid is chosen from the group consisting of the amino acids between position 171 and position 180 of the sequence shown in Fig. 15 (SEQ ID NO: 12), inclusive. A construct exemplified herein ends with a carboxyterminal residue at position 171, so that the mutant core protein includes amino acids

1-171 (Fig. 15 (SEQ ID NO: 12)). In another example, the carboxyterminal amino acid is amino acid 178, so that the mutant core protein includes amino acids 1-178 (Fig. 15 (SEQ ID NO: 12)), corresponding to a five amino acid deletion from the carboxyterminus (see, e.g., the analogous duck hepatitis B virus (DHBV) construct pBK, which is described below). The first amino acid sequence is at least 70 amino acids in length, e.g., 72, 74, 76, 78, or 80 amino acids in length. The aminoterminal amino acid of the first amino acid sequence can be the first amino acid of the corresponding wild type hepadnavirus sequence. Alternatively, nonessential aminoterminal amino acids can be eliminated from the mutant polypeptide, provided that the resulting mutant polypeptide does not lose substantial inhibitory activity as a result, when tested according to the methods described below.

By "lacks a second amino acid sequence" is meant that at least three amino acids from the carboxyterminal end of the core protein have been deleted to make the mutant. Preferably, the deleted sequence includes amino acids 171-183 of the HBV core protein; i.e., the second amino acid sequence includes amino acids 171-183 of the sequence shown in Fig. 15 (SEQ ID NO: 12), inclusive.

In another embodiment of the method of inhibiting hepadnavirus replication, the mutant polypeptide further includes a third amino acid sequence. The third amino acid sequence is substantially identical to a portion of a wild type hepadnavirus surface protein. The aminoterminal amino acid of the third amino acid sequence may be joined by a peptide bond to the carboxyterminal amino acid of the first amino acid sequence so as to create a fusion protein. The third amino acid sequence can be the entire surface protein, or can be a portion thereof, e.g., a portion of at least 4, 8, 20, 30, or 43

amino acids in length. For example, the aminoterminal amino acid of the third amino acid sequence can be selected from the group consisting of the amino acids between position 1 and position 112 of the sequence shown in Fig. 16 (SEQ ID NO: 14), inclusive, preferably the amino acids between position 1 and position 8, inclusive. Preferred aminoterminal amino acids of the third amino acid sequence exemplified herein include, but are not limited to, position 5 or position 8 of Fig. 16 (SEQ ID NO: 14).

The carboxyterminal amino acid of the third amino acid sequence can be selected from a group that includes any of the amino acids between position 51 and position 224 of Fig. 16 (SEQ ID NO: 14), inclusive; e.g., any of the amino acids between position 112 and position 224 of Fig. 16 (SEQ ID NO: 14), inclusive; e.g., the carboxyterminal amino acid may be position 51, position 112, or position 224 of Fig. 16 (SEQ ID NO: 14). Thus, the portion of the surface protein included on the mutant polypeptide preferably includes surface protein residues 1-112, 8-112, or 8-51, all inclusive (Fig. 16; SEQ ID NO: 14).

The use of a core protein for inhibiting viral replication is a species-specific event, so that mutant core proteins inhibit nucleocapsid assembly in the same type of hepadnavirus from which they were derived. Thus, the first amino acid sequence is substantially identical to a region of a wild type hepadnavirus core protein that is derived from the same type of hepadnavirus (e.g., HBV versus DHBV) as the naturally-occurring hepadnavirus targeted for inhibition. In contrast, the third amino acid sequence may be substantially identical to a portion of a wild type hepadnavirus surface protein of any hepadnavirus species, since the surface proteins do not demonstrate species specificity. Thus, when the method

of the invention is used to treat an HBV infection, the mutant polypeptide should include sequences specifically derived from the HBV core protein Fig. 15 (SEQ ID NO: 12), but can include sequences derived from any species of surface protein (e.g., the sequence of Fig. 16 (SEQ ID NO: 14)).

In another embodiment, the invention features a nucleic acid that encodes a mutant hepatitis B virus (HBV) polypeptide, the polypeptide including a first amino acid sequence that is substantially identical to a region of a wild type HBV core protein, and lacking a second amino acid sequence of the wild type HBV core protein. The second sequence includes the carboxyterminal three amino acids of the wild type HBV core protein and does not exceed nine amino acids in length. Thus, the carboxyterminal amino acid of the first amino acid sequence can be at position 174, position 175, position 176, position 177, position 178, position 179, or position 180, all of Fig. 15 (SEQ ID NO: 12).

In another embodiment, the invention features a nucleic acid that encodes a mutant hepadnavirus polypeptide. The polypeptide includes a first amino acid sequence that is substantially identical to a region of a wild type hepadnavirus core protein; lacks a second amino acid sequence of the wild type hepadnavirus core protein which includes at least the carboxyterminal three amino acids of the wild type hepadnavirus core protein; and includes a third amino acid sequence that is substantially identical to a portion, or all, of a wild type hepadnavirus surface protein. The aminoterminal amino acid of the third amino acid sequence may be joined by a peptide bond to the carboxyterminal amino acid of the first amino acid sequence so as to create a fusion protein. The carboxyterminal amino acid of the first

amino acid sequence can be any of the amino acids between position 71 and position 180 of Fig. 15 (SEQ ID NO: 12), inclusive. Preferably, the second amino acid sequence does not exceed 100 amino acids in length.

The invention also features polypeptides encoded by any of the various nucleic acids of the invention. A polypeptide of the invention can be included in a therapeutic composition as an active ingredient, along with a pharmaceutically acceptable carrier, or it can be expressed from the nucleic acid within the infected cell.

The invention also features vectors into which are inserted any of the various nucleic acids of the invention. The vector can include any sequence known to those of skill in the art necessary or desirable for replicating the vector in a eukaryotic cell or for expressing a polypeptide of the invention from the coding sequences thereon. For example, the nucleic acid sequence can be operatively linked to appropriate transcription and/or translation control sequences that function in a eukaryotic cell. The vector can be any vector suitable for maintaining or making multiple copies of a nucleic acid of the invention, or can be one that is suitable for administering a nucleic acid of the invention to a cell or to a mammal infected with a hepadnavirus, e.g., to a human patient infected with HBV or to cells removed from the patient for ex vivo gene therapy. Examples of vectors useful in the method of inhibiting a hepadnavirus include, but are not limited to, adenovirus vectors, adeno-associated vectors, and retroviral vectors. Any of the various vectors of the invention can be included in a therapeutic composition along with a pharmaceutically acceptable carrier.

In another aspect the invention includes a method of evaluating a candidate polypeptide for its ability to inhibit the replication of a naturally-occurring

hepadnavirus. The method involves introducing a mutant hepadnavirus polypeptide as described above into a medium in the presence of the hepadnavirus and determining whether hepadnavirus replication is inhibited in the presence of the polypeptide, compared to in its absence, such inhibition being an indication that the polypeptide is an inhibitor of hepadnavirus replication. By "medium" is meant an environment that is capable of supporting viral replication by virtue of its chemical composition. The medium can be within an organism, e.g., an animal model, or can be within an organ removed from an animal. The medium can also be an intracellular medium, e.g., in a cell culture assay, or a cell-free extract, e.g., a cell free replication system. Examples of cells suitable for a cell culture assay include, but are not limited to, Huh-6, Huh-7, HepG2, HepG2 2215, LMH, DC, and HCC cells. The polypeptide can be introduced to the medium by introducing into the medium a nucleic acid encoding the polypeptide, with subsequent expression of the polypeptide therein.

Another method of inhibiting the replication of a naturally-occurring hepadnavirus involves introducing into the proximity of the hepadnavirus a hepadnavirus mutant polypeptide, or a nucleic acid that encodes a hepadnavirus mutant polypeptide. The polypeptide includes a first amino acid sequence that is substantially identical to a region of, or all of, a wild type hepadnavirus core protein, and a second amino acid sequence which is substantially identical to a portion of, or all of, a wild type hepadnavirus surface protein. The aminoterminal amino acid of the second amino acid sequence may be joined by a peptide bond to the carboxyterminal amino acid of the first amino acid sequence so as to create a fusion protein. The second amino acid sequence can be the entire surface protein, or

can be a portion thereof. The mutant polypeptide is expressed from the nucleic acid in the proximity of the naturally-occurring hepadnavirus, so as to be available to inhibit replication of the hepadnavirus.

In a final aspect, the invention includes a hepadnavirus mutant polypeptide, or a nucleic acid that encodes a hepadnavirus mutant polypeptide. The polypeptide includes a first amino acid sequence that is substantially identical to a region, or all, of a wild type hepadnavirus core protein, and a second amino acid sequence which is substantially identical to a portion, or all, of a wild type hepadnavirus surface protein. The aminoterminal amino acid of the second amino acid sequence may be joined by a peptide bond to the carboxyterminal amino acid of the first amino acid sequence so as to create a fusion protein. The second amino acid sequence can be the entire surface protein, or can be a portion thereof.

As used herein, a "hepadnavirus" refers to a member of the hepadnavirus family of viruses, including, but not limited to, hepatitis B virus and hepatitis delta virus (Wang et al., Nature, 323:508-13, 1986). Although treatment of HBV is an important feature of the method of invention due to the incidence of HBV-related human disease, the methods described herein also apply to other species of hepadnaviruses. Examples of hepadnaviruses within the scope of the invention include, but are not limited to, hepadnaviruses infecting various human organs, including liver cells, exocrine and endocrine cells, tubular epithelium of the kidney, spleen cells, leukocytes, lymphocytes, e.g., splenic, peripheral blood, B or T lymphocytes, and cells of the lymph nodes and pancreas (see, e.g., Mason et al., Hepatology, 9:635-645, 1989). The invention also applies to hepadnaviruses infecting non-human mammalian species, such as

domesticated livestock or household pets. In addition, the invention includes a method of evaluating a candidate mutant polypeptide for its ability to inhibit hepadnavirus replication. For the purposes of conducting a laboratory screening assay, a variety of hepadnavirus species are useful models. Examples include, but are not limited to, woodchuck hepatitis virus (WHV; Summers et al. Proc. Natl. Acad Sci. USA, 75:4533-37, 1978), duck hepatitis B virus (DHBV; Mason et al. J. Virol. 36:829-36, 1978), and squirrel hepatitis virus (e.g., Marion et al. Proc. Natl. Acad Sci. USA, 77:2941-45, 1980).

Although particular amino acids are referred to below with reference to the sequence of HBV (Figs. 15 and 16; SEQ ID NOs: 11-14), it is understood that the invention encompasses mutant polypeptides comprising corresponding amino acid segments derived from other hepadnavirus species. One of ordinary skill in the art can easily compare closely-related sequences to locate the analogous amino acid positions in related hepadnaviruses; the descriptions provided in Examples 2 and 3 illustrate examples of such comparisons.

Where the method of inhibiting hepadnavirus replication is used to treat a hepadnaviral infection in an animal, a "naturally-occurring" hepadnavirus refers to a form or sequence of the virus as it exists in an animal, e.g., a natural isolate derived from an infected animal. In all other contexts, a "naturally-occurring" hepadnavirus is intended to be synonymous with the sequence known to those skilled in the art as the "wild type" sequence, e.g., the wild type HBV core and surface protein sequences shown in Figs. 15 and 16 (SEQ ID NOs: 11-14). If an amino acid sequence of a core or surface protein of a hepadnavirus that is derived from a natural isolate differs from the conventionally accepted "wild type" sequence, it is understood that the sequence of the

natural isolate may be the proper comparison sequence for designing mutant polypeptides of the invention.

"Sequence identity", as used herein, refers to the subunit sequence similarity between two nucleic acid or polypeptide molecules. When a given position in both of the two molecules is occupied by the same nucleotide or amino acid residue, e.g., if a given position in each of two polypeptides is occupied by serine, then they are identical at that position. The identity between two sequences is a direct function of the number of matching or identical positions, e.g., if half (e.g., 5 positions in a polymer 10 subunits in length) of the positions in two polypeptide sequences are identical, then the two sequences are 50% identical; if 90% of the positions, e.g., 9 of 10, are matched, the two sequences share 90% sequence identity. Methods of sequence analysis and alignment for the purpose of comparing the sequence identity of two comparison sequences are well known by those skilled in the art. By "substantially identical" is meant sequences that differ by no more than 10% of the residues, and only by conservative amino acid substitutions such as those shown in Table 1, or non-conservative amino acid substitutions, deletions, or insertions that do not appreciatively diminish the polypeptide's biological activity, e.g., an insertion of amino acids at the junction of the core protein and surface protein sequences that has no appreciative effect on biological activity. "Biological activity", as used herein, refers to the ability of a mutant polypeptide to inhibit hepadnavirus replication, and can be measured by the assays described below.

Other terms and definitions used herein will be understood by those of routine skill in the art. For example, by "inhibiting the replication of" is meant lowering the rate or extent of replication relative to

replication in the absence of a mutant polypeptide of the invention. By "into proximity with the hepadnavirus" is meant introducing into a cell, organ, or organism which is infected with a naturally-occurring hepadnavirus, or, in the case of laboratory application, cotransfection or co-inoculation with a wild type hepadnavirus. By "nucleic acid" is meant deoxyribonucleic acid (DNA) or ribonucleic acid (RNA).

The methods, nucleic acids, and polypeptides of the invention can be used to inhibit hepadnaviral replication in a mammal, e.g., as an effective therapy for treating individuals with a persistent HBV infection, or as a means of reducing the risk of hepatocellular carcinoma in an infected animal. Polypeptides of the invention can be administered to an infected animal either directly or by gene therapy techniques. The screening methods of the invention are simple, rapid, and efficient assays designed to identify polypeptides with anti-hepadnaviral activity.

Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

Brief Description of the Drawings

Fig. 1 is a schematic illustration of the structural organization of "wild type" and mutant hepadnavirus constructs.

Fig. 2 is an autoradiographic image of an agarose gel, showing a Southern blot analysis of core particle DNA that was extracted from HuH-7 cells five days post transfection and probed with full length ^{32}P-labeled WHV DNA.

Fig. 3 is an autoradiographic image of an agarose gel, showing a Southern blot analysis of core particle associated viral DNA that was extracted from HuH-7 cells

five days after transfection and probed simultaneously with the two full length ^{32}P-labeled WHV and HBV DNA probes.

Fig. 4 is an autoradiographic image of an agarose gel, showing a Southern blot analysis of core particle associated viral DNA that was extracted from HepG2 cells five days after transfection and probed with full length ^{32}P-labeled HBV DNA.

Fig. 5 is an autoradiographic image of a polyacrylamide gel showing a RNase protection assay.

Fig. 6 is an autoradiographic image of an agarose gel showing a Southern blot analysis of the anti-viral effect of dominant negative core mutants on "wild type" HBV replication during transient transfection in HuH-7 cells.

Fig. 7 is an autoradiographic image of a SDS-polyacrylamide gel showing a Western blot analysis of HepG2 cell lysates probed with anti-HBc antibodies.

Fig. 8 is an autoradiographic image of an agarose gel showing a Southern blot analysis of the effect of a dominant negative core mutant on replication of HBV in Hep-G2 2215 cells.

Fig. 9 is an autoradiographic image of an agarose gel showing a Southern blot analysis of cytosol-derived nucleocapsids from transfected LMC cells hybridized with a full length DHBV DNA probe.

Fig. 10 is an illustration of the nucleic acid sequence of the pCN4 plasmid insert (SEQ ID NO: 1) and the corresponding translated amino acid sequence (SEQ ID NO: 2).

Fig. 11 is an illustration of the nucleic acid sequence of the pHBV DN plasmid insert (SEQ ID NO: 3) and the corresponding translated amino acid sequence (SEQ ID NO: 4).

Fig. 12 is an illustration of the nucleic acid sequence of the pHBV DN AA plasmid insert (SEQ ID NO: 5) and the corresponding translated amino acid sequence (SEQ ID NO: 6).

Fig. 13 is an illustration of the nucleic acid sequence of the pHBV DN BB plasmid insert (SEQ ID NO: 7) and the corresponding translated amino acid sequence (SEQ ID NO: 8).

Fig. 14 is an illustration of the nucleic acid sequence of the pDHBV BK plasmid insert (SEQ ID NO: 9) and the corresponding translated amino acid sequence (SEQ ID NO: 10).

Fig. 15 is an illustration of the nucleic acid sequence of the HBV core protein (SEQ ID NO: 11) and the corresponding translated amino acid sequence (SEQ ID NO: 12).

Fig. 16 is an illustration of the nucleic acid sequence of the HBV core protein (SEQ ID NO: 13) and the corresponding translated amino acid sequence (SEQ ID NO: 14).

Detailed Description

Applicants have observed that replication of a wild type hepadnavirus is reduced when it is co-transfected with a nucleic acid construct encoding a truncated core protein, or a core-surface fusion protein. The truncated core protein, alone or in combination with the surface protein component, has a deletion of at least three amino acids from the carboxyterminal end. Viral replication is reduced by as much as 90-95% without detectable toxic effects on the host cell. Constitutively expressing a HBV mutant core-surface fusion protein as a retroviral insert substantially inhibits HBV viral DNA production in cells that previously had continuously produced all viral

replicative intermediates and infectious virions. An adenoviral-based plasmid that encodes the same mutant core-surface fusion protein also inhibits HBV replication following transient cotransfection in HCC cells. These dominant negative effects on viral replication are consistent over a range of hepadnavirus species.

Materials and Methods.

Materials and methods useful for practicing the invention are described as follows:

Plasmid Constructs. The parental plasmid pCMW82 was used to generate a series of constructs expressing WHV core proteins with an altered carboxyterminal region. Plasmid pCMW82 expresses the "wild type" WHV pregenome under the control of the cytomegalovirus immediate-early (CMV IE) promoter (Seeger et al., *J. Virol.*, 63, 4665-4669, 1989). The pHBV plasmid carries the HBV pregenome under the control of the CMW IE promoter. These plasmids direct the synthesis of complete virions in tissue culture cells. The first nucleotide of the precore open reading frame was designated as nucleotide number 1 in the WHV genome.

The structural organization of "wild type" and mutant WHV, HBV, and DHBV core plasmid constructs are depicted in Fig. 1. The white boxes represent the open reading frame (ORF) used for constructing core mutants. Numbers at the boundaries of each ORF refer to the amino acids in the "wild type" or mutant proteins. Dotted lines represent deleted sequences. Solid and hatched boxes correspond to mutant core proteins expressed from WHV and HBV, respectively. Shaded bars refer to DHBV. The shaded hatched bars refer to the polymerase gene. Except for the "wild type" constructs pCMW82 and pCMW-DHBV, all other constructs are incapable of replication because of deletions in genes that overlap the truncated

portions of the core protein. The * refers to a stop codon introduced by a frame shift mutation.

The constructs shown in Fig. 1 were produced by complete digestion with the appropriate restriction enzyme. This was followed by subsequent incubation at 30°C for 20 min. in the presence of the Klenow fragment of DNA polymerase I and deoxyribonucleotide triphosphates, which filled in the 3' recessed DNA ends. Plasmids were then ligated with T4 DNA ligase. The 3' protruding ends were filled in by incubation with the Klenow fragment of DNA polymerase I in the absence of deoxyribonucleotide triphosphates at 37°C for 15 min. This eliminated protruding ends. Deoxyribonucleotide triphosphates were then added and incubation was carried out at 30°C for 20 min. (Sambrook et al., Molecular Cloning: A Laboratory Manual, *Cold Spring Harbor Laboratory*, Cold Spring Harbor, NY, 1989).

Constructs containing mutations in sequences encoding the core protein were obtained as follows: 1) pCN1: To make the plasmid pCN1 the WHV core gene was digested with the restriction enzyme SstI at nucleotide (nt) 310, incubated with Klenow DNA polymerase, and reclosed with T4 DNA ligase. This introduced a frame shift mutation at nt. 306 in the WHV core gene, thereby creating a stop codon at nt. 317. This mutation produces a 74 amino acid carboxyterminal truncated core protein, leaving intact the rest of the viral coding regions. 2) pCN2: To make pCN2 the WHV parental plasmid was digested with the restriction enzymes BglII (nt. 601 in the core gene) and SmaI, the latter being located in the downstream multiple cloning site of the vector. The intervening viral genes were separated by gel electrophoresis, and the DNA ends were filled in with Klenow DNA polymerase and ligated with T4 DNA ligase. This WHV core gene has 12 amino acids deleted at the

carboxyterminus and is fused to a three amino acid heterologous extension from the plasmid vector. 3) pCN3: To make the plasmid pCN3, the wild type plasmid pCMW82 was digested with the restriction enzymes BglII and SacII (position 2983 in the WHV X gene), the intervening viral DNA fragment was removed, and ends were filled in and ligated. The resulting plasmid construct encodes a 171 amino acid core protein fragment fused in-frame with the X protein at amino acid 31. 4) pCN4: The plasmid pCN4 was produced by a BglII-MscI (position 1826) fragment excised from pCMW82 and blunted by Klenow DNA polymerase. The plasmid was ligated to join the WHV 171 amino acid core protein as an in-frame fusion protein with amino acid 47 of the WHV small surface protein. 5) pCN5: The plasmid pCN5 was produced by removing the DNA fragment SstI (pos. 306)-BspEI (pos. 519) from pCN4, and blunting the ends with Klenow DNA polymerase and T4 DNA ligase. This introduced a WHV core in-frame deletion between amino acids 74 and 145. 6) Plasmid pCN6 expresses the first 171 amino acids of the WHV core protein fused in-frame with the HBV small surface protein at amino acid 51.

The HBV numbering system designates the unique EcoRI site as nucleotide 1. Construct pHBV DN was generated by digesting pCMW82 at nt. 601 of the core gene with BglII, and blunting the DNA end with Klenow DNA polymerase. A second cut was performed with PvuI in the ampicillin resistance gene of the carrier plasmid, and the BglII-PvuI DNA fragment was removed by fractionation on an agarose gel. The HBV MscI (pos. 299)-PvuI (in the ampicillin resistance gene of pHBV) fragment was ligated to the blunted BglII-PvuI fragment.

In order to produce an in-frame dominant negative construct of HBV that was similar to the pCN4 WHV construct, the pCN6 fragment from the SnaBI site (which

cuts in the CMV promoter of the carrier plasmid) to the BspEI site (pos. 519 in the WHV) was removed and substituted with the same SnaBI-BspEI (pos. 2327) fragment from pHBV. In this way, the HBV core protein was fused in-frame to amino acid 144 of the WHV core protein. This fragment, derived from plasmid pCN6, was already fused at amino acid 171 to the HBV small surface protein at amino acid 51. The resulting pHBV DN therefore encodes, in the hinge between the deleted core and surface proteins, five amino acids derived from the WHV core protein (GGARA). These five amino acids were not present in the subtype HBV core protein. The carboxyterminal 20 amino acid of the WHV core protein are conserved in HBV.

Two additional plasmids were derived from pHBV and called pHBV AA and pHBV BB. To make pHBV DN AA, pHBV was partially digested with the restriction enzyme AvaI (nt. 2431), and then partially digested with AvrII (nt. 176). The resulting DNA ends were blunted by adding Klenow DNA polymerase and nucleotide triphosphates. The DNA ends were ligated with T4 ligase. The resulting plasmid pHBV DN AA encodes the HBV core protein fused in frame at amino acid 179 with the surface protein (encoded by the "S gene") at amino acid 8. The plasmid pHBV BB was made by performing two sequential partial digestions with the enzymes BglII and BamHI. The DNA ends were ligated with T4 ligase. The pHBV BB plasmid expresses the HBV core protein fused in frame at amino acid 175 with the surface protein at amino acid 112. The correct design of the constructs was confirmed by restriction digest mapping and DNA sequence analysis. Plasmid DNAs were purified by the alkali lysis procedure followed by sedimentation through a cesium chloride-ethidium bromide density gradient. As a result of these viral gene manipulations the above plasmid constructs produce replication

defective WHV genomes. Plasmid pCN1 expresses a truncated core protein that is unable to assemble into functional nucleocapsids. All other constructs contain inactivating deletions in the polymerase gene.

Another plasmid, designated pRHBBE, was constructed using the polylinker of the plasmid pBS SK(+)(Stratagene), which allows for viral gene transcription from the T7 promoter to make a HBV-specific 276 nt antisense RNA. This species, encoded by a BamHI (pos. 2906) to EcoRI (pos. 1) fragment, was used in RNase protection experiments. The ^{32}P labeled riboprobe annealed specifically to the "wild type" pregenomic HBV DNA without recognizing the pHBV DN mRNA.

Constructs expressing DHBV dominant negative proteins were derived from the plasmid pCMV DHBV (Wu et al., *J. Virol.*, 65, 2155-2163, 1991), which expresses the DHBV pregenome under the control of the CMV promoter. Construct pSK contains a deletion between the SphI site (position 2843 in the core gene; this numbering system is arbitrarily initiated with the nucleotide GAATTC of the unique EcoRI site) and the KpnI site (position 1290, in the S gene). The intervening fragment was separated by agarose gel electrophoresis. The ends of the larger DNA fragment were blunted by Klenow DNA polymerase and religated. This construct expresses, under the control of the CMV promoter, a protein composed of the first 66 amino acids of the DHBV core protein fused in frame to amino acid five of the DHBV surface protein. Construct pBK contains a deletion between the BglII site (position 391 in the core gene) and the KpnI site (position 1290 in the S gene). The intervening fragment was separated by agarose gel electrophoresis and the ends of the larger DNA fragment were filled in and blunted by the Klenow DNA polymerase. The ends were then religated. The resulting construct expresses, under the control of the CMV

promoter, a protein composed of the first 257 amino acids of the DHBV core protein (five amino acids are missing from the carboxyterminus), fused in frame to the fifth amino acid of the DHBV surface protein.

To make the construct pK, the pCMV DHBV was linearized by cutting at the KpnI site (position 1290 of the S gene). The DNA ends were blunted with the Klenow DNA polymerase reaction and the fragment was religated. The resulting construct has a frame-shift mutation so that the DHBV polymerase pK gene and the pre-S and S genes have a termination site a few nucleotides downstream from the KpnI site. The construct pK thus expresses, under the control of the CMV promoter, the full length core protein, but none of the envelope proteins apart from a truncated pre-S protein. A frameshift mutation that occurs in the polymerase gene renders the other constructs carrying the deletions described above replication defective. Construct pNX contains a deletion between the NsiI site (position 2845 in the core gene) and the XhoI site (position 1212 in the pre-S gene). The intervening fragment was separated by agarose gel electrophoresis. The ends of the larger DNA fragment were blunted and filled in with Klenow DNA polymerase, followed by religation of the fragment to itself. This construct expresses, under the control of the CMV promoter, the first 68 amino acids of the DHBV core protein fused in frame to amino acid 437 of the carboxyterminus of the polymerase gene.

Retroviral constructs: The HBV core-surface fusion gene encoded by pHBV DN was PCR amplified with oligonucleotides containing at their 5′ ends a SalI restriction enzyme recognition site. The antisense primer contained a recognizable Flag epitope (Kodak). The PCR product was gel purified, digested with SalI, and cloned in the retroviral pBabe Puro vector (Morgenstern

et al., *Nucl. Acids Res.*, 18:3587-96, 1990) at its SalI site. The design of the resulting pBP HBV DN vector was confirmed by sequence analysis.

Transfections into Hepatoma Cell Lines: Human hepatoma cells HuH-7 and HepG2 support a complete viral replicative cycle following transfection with a plasmid construct expressing the pregenomic viral RNA (Mason et al., *Hepatology*, 9, 635-45, 1989). Cells were maintained and passaged as previously described (Wu et al., *J. Virol.*, 65, 2155-2163, 1991). Cells were transiently co-transfected with plasmids expressing the mutated WHV or HBV core genes (described above), together with an equal amount of a "wild type" WHV or HBV producing plasmid. Co-transfections were performed by the calcium phosphate technique ($CaPO_4$ transfection Kit, 5′-3′, Boulder, Colorado). Briefly, 1.2 x 10^7 cells in 100 mm plates were grown for 24 hours. The medium was changed 2-4 hours before transfecting with 10 μg of "wild type" virus. This produces the plasmid along with 10 μg of each mutant construct. The precipitate was left on the cells for 6-8 hours, and then the medium was replaced. The cells were harvested two days after transfection when performing RNA experiments, and five days after transfection when performing DNA experiments.

The cell line LMH, derived from a chicken hepatocellular carcinoma, was used for transfection of the DHBV derived plasmids. This cell line supports higher levels of DHBV replication than do cell lines of human origin. Another cell line, derived from LMH and named D2, was created by stably transfecting a head-to-tail DHBV dimer that produces infectious DHBV particles. These cells were grown in DMEM and 10% FCS and transfected with the various dominant negative core mutant constructs as described above.

Infection of the HepG2 2215 Cell Line: Infection of the HepG2 2215 cell line by recombinant retroviruses was accomplished following a standard protocol for producing retroviral stocks and for infecting tissue culture cells (Miller et al., *Biotechniques*, 7, 980-990, 1989; Miller, et al., Methods in Enzymology, 217, 581, 1993). After infection, the cells were selected with 2 μg/ml puromycin (Sigma). Resistant clones were pooled and further expanded.

Analysis of viral DNA replication. WHV and HBV DNA replication were assayed by Southern blot analysis of DNA that had been extracted from intracellular core particles. The procedure for isolation of core particles was previously described in detail (Pugh et al., *J. Virol.*, 62, 3513-3516, 1988). DNA fractionation on agarose gels was performed under alkali conditions and the DNA was transferred onto a nylon membrane for Southern blot analysis (Hybond N, Amersham International, Little Chalfont, UK). Prehybridization and hybridization reactions were carried out at 65°C in 6X SSC solution (1X SSC is 150 mM NaCl, 15 mM Na_3Citrate), 5X Denhardt's solution (100X is 2% w/v BSA, 2% w/v Ficoll, 2% w/v polyvinyl pirollidone), and 0.5% SDS. WHV and HBV DNAs were detected by hybridization with randomly primed ^{32}P-labeled full length WHV or HBV DNA (Multiprime DNA Labelling System, Amersham). The membranes were washed twice for 15 min. each at 65°C in 1X SSC, 0.1% SDS, and were then washed once more at 65°C in 0.1X SSC, 0.1% SDS. The nylon membranes were then autoradiographed at -70°C, using intensifying screens and Kodak films. Signal intensities on the nylon sheets were quantitated by a computer assisted scanning system (Ambis Quantprobe System version 3.0).

Extraction and analysis of viral RNA. Total RNA was extracted from a 100 mm dish by lysis of cells in 1

ml of solution D (4 M guanidinium thiocyanate, 25 mM NaCitrate, pH 7.0, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol) as described (Chomczynski et al., Anal. Biochem., 168, 156-159, 1987). Encapsidated viral RNA was extracted from core particles by lysis in 200 ml of solution D and the volumes were adjusted accordingly as described (Roychoury et al., *supra*). Finally, to exclude contamination by plasmid DNA or reversed transcribed HBV DNA, the encapsidated viral RNA was digested with 16 U RNase-free DNase RQ1 DNase (Promega, Madison, WI) at 37°C for 15 min., followed by phenol-chloroform extraction and ethanol precipitation, before undergoing the RNase protection assay.

RNase protection analysis of total and encapsidated viral RNA was performed with a commercially available kit according to the manufacturer's instructions (RPA II-Ribonuclease protection kit, Ambion Inc. Austin, TX). The RNA probe was derived from the plasmid pRHBBE, a derivative of the pBluescript SK(+), which includes the 280 bp HBV fragment BamHI (pos. nt 2901)-EcoRI (pos. nt 1), oriented to produce an antisense RNA molecule when transcription was initiated with the bacteriophage T7 RNA polymerase. The RNA probe contained approximately 50 nt of plasmid sequences that were not protected by the HBV specific RNA. Labeled RNA was synthesized as follows: 0.5 μg of pRHBBE was cut by BamHI and then transcribed by T7 RNA polymerase (Promega, Madison, WI) in the presence of α-^{32}P UTP (100 μCi at 400 Ci/mM, New England Nuclear, Boston, MA). The antisense RNA probe recognized pregenomic RNA and the 2.4 pre-S1 mRNA derived from "wild type" HBV, but did not recognize transcripts derived from pHBV DN. Hybridization, after denaturation at 95°C for 3 min., was performed in 20 μl on 2 μg of total RNA or encapsidated pregenomic RNA derived from half of a 100 mm plate at 42°C overnight in

a solution of 80% formamide, 100 mM NaCitrate pH 6.4, 300 mM NaAcetate pH 6.4, and 1mM EDTA. RNase digestion was carried out with RNase A (0.5U) and RNase T1 (20 U) at 37°C for 30 min. Fragments protected by RNase digestion were separated on a denaturing 6% polyacrylamide gel (Sequagel 6%, National Diagnostics, Atlanta, GA).

Viral nucleocapsid isolation and Western blots. HepG2 cells that had been transfected with pHBV alone, pHBV DN together, or pHBV DN alone were lysed in 500 ml TNE, 1% NP 40. The debris was pelleted by centrifugation at 10,000 rpm in an Eppendorf bench top centrifuge. A 200 µl aliquot of the clarified cell lysate was ultracentrifuged at 500,000 xg for 1 hour at 4°C through 2 ml of a 20% w/v sucrose/TNE cushion using a TLA 100 rotor (Beckman Instruments, Palo Alto, CA). Under these conditions viral core particles were pelleted, whereas free core protein and soluble hepatitis Be antigen (HBeAg) remained in the supernatant (Zhou et al., *supra*). The pellet was resuspended in 100 µl of Laemmli sample buffer and boiled for 3 min. One-half of the sample was run over a 12.5% SDS-PAGE gel (Acrygel National Diagnostics, Atlanta, GA). Western blotting was performed on an Immobilon-P membrane (Millipore Co., Bedford, MA) (Harlow et al., Antibodies: a laboratory manual, *Cold Spring Harbor Laboratories*, CSH, NY 1988). After transfer the membrane was blocked for one hour in a solution of 5% non-fat dry milk and 0.5% Tween-20 in phosphate buffered saline (PBS). HBcAg antigenicity was detected by incubation of the membrane with polyclonal antibodies prepared in rabbits against recombinant HBcAg (Dake Co., Carpinteria, CA) at a 1:250 dilution in the above solution for one hour at 20°C. The filter was washed at 20°C in PBS, 0.5% Tween-20 with three successive changes of solution. Bound antibody was detected using the chemiluminescence method (ECL,

Amersham International, Little Chalfont, UK) using peroxidase labeled goat anti-rabbit antibodies. The filter was exposed to Kodak films for 5-20 seconds.

Experimental Results

Inhibition of WHV DNA synthesis. WHV core mutant plasmids were tested for the ability to inhibit wild type WHV DNA replication in HuH-7 cells. Fig. 2 shows a Southern blot analysis of core particle DNA that was extracted from HuH-7 cells five days post transfection and probed with full length ^{32}P labeled WHV DNA. Lane M contains ^{32}P 5' end labeled lambda HindIII molecular weight markers. The HuH-7 cells were transfected with: lane 1, pCMW82; lane 2, pCMW82 and pCN4; lane 3, pCMW82 and pCN1; lane 4, pCMW82 and pCN2; lane 5, pCMW82 and pCN3; and lane 6, pCMW82 and pCN5. Each lane was loaded with one-half of the core associated viral DNA, which had been extracted from a 100 mm tissue culture dish of HuH-7 cells.

All mutant WHV core constructs suppressed "wild type" WHV DNA synthesis, albeit with different efficiencies. The extent of inhibition varied among the different constructs, depending in part on the molecular structure of the mutant core protein. In order to exclude experimental variability, all transfections were repeated several times with comparable results. The data represent an average of at least three independent experiments. Cotransfection of "wild type" pCMW82 with the mutant core constructs pCN1, pCN2, and pCN3 produced a modest inhibition of "wild type" viral DNA replication (36%, 48%, and 12%, respectively). In contrast, pCN4 and pCN5 substantially inhibited WHV DNA synthesis in HuH-7 cells by 90% and 85%, respectively (Fig. 2).

To test whether the pCN4 construct inhibits HBV replication, cotransfection experiments were performed

with "wild type" pHBV. There was no reduction of HBV synthesis by the WHV based construct pCN4. Fig. 3 shows a Southern blot analysis of core particle associated viral DNA extracted from HuH-7 cells five days after transfection. The blots were probed simultaneously with full length ^{32}P labeled WHV and HBV DNA probes. Lane M contains ^{32}P 5′ end labeled lambda HindIII molecular weight markers. Core particle associated viral DNA was extracted from cells transfected with: lane 1, pCMW82; lane 2, pCMW82 and pCN4; lane 3, pCMW82 and pCN6; lane 4, pHBV; lane 5, pHBV and CN4; and lane 6, pHBV and pCN6. Each lane was loaded with one-half of the core particle associated DNA that had been extracted from a 100 mm tissue culture dish of HuH-7 cells.

In order to determine the general region of the fusion protein that was responsible for inhibiting viral replication, a chimeric construct expressing WHV core-HBV small surface fusion protein was produced. This plasmid, designated pCN6, reduced "wild type" WHV replication by 85%, an inhibitory effect comparable to the original parental construct pCN4. Like pCN4, pCN6 does not inhibit HBV replication (Fig 3, lane 6). It was concluded that the WHV core-small surface fusion protein encoded by pCN4 exerts a species-specific inhibitory effect.

To determine the amount of pCN4 required to interfere effectively with WHV replication, HuH-7 cells were co-transfected at various ratios of CMW82 to pCN4 using 10 μg of pCMW82. The total amount of transfected DNA was kept constant (20 μg) by adding unrelated sonicated salmon sperm DNA. The results of these experiments indicate that when pCN4 was diluted by 10 and 50 fold, there was still a 66% and 20% inhibition of "wild type" WHV replication, respectively. Interference

with viral replication occurs even in the presence of an excess of "wild type" core protein.

Dominant negative core mutant polypeptides are not toxic to HCC cells. To insure that the mutant plasmids were neither affecting the efficiency of DNA uptake by HuH-7 cells during transfection, nor inducing a cytopathic effect, each 100 mm plate had a 10 mm cover slip containing cells grown under the same conditions. The cells were investigated by immunocytochemistry utilizing the protocol of Jilbert et al. (*J. Virol.*, 66, 1377-1378, 1992). Core protein expression was detected with polyclonal antibodies prepared against either WHV or HBV recombinant core proteins. Approximately one percent of the HuH-7 cells were transfected with the "wild type" WHV plasmid, as demonstrated by diffuse cytoplasmic staining for WHcAg in cells harvested five days post transfection. After transfection of cells with pCN4 alone, a punctate distribution of WHcAg in the perinuclear region was observed. The same staining pattern was obtained when the dominant negative core mutant constructs were co-transfected with "wild type" pCMW82. The total number of HBcAg positive cells did not vary under these conditions. The mutant core expressing plasmids did not inhibit "wild type" viral DNA uptake during the transfection process and were not toxic to HuH-7 cells.

It was also necessary to exclude the possibility that the inhibitory effect exerted by pCN4 on WHV replication was the result of decreased transcription of "wild type" WHV pregenomic RNA. For these studies, Poly(A)$^+$RNA was extracted from HuH-7 cells that had been transfected with the plasmids pCMW82 alone, pCMW82 and pCN4 together, or pCN4 alone. The RNA was probed with a BglII-BstXI WHV DNA fragment that specifically recognized the pregenomic RNA but not the pCN4 transcripts. The

results demonstrated no change in the level of "wild type" WHV pregenomic RNA transcription from pCMW82 in the presence of pCN4.

Inhibition of HBV replication. Based on the previous results, it was of interest to determine whether a similar mutant core polypeptide would inhibit HBV replication in HCC cells. The construct pHBV DN was designed to be the molecular HBV-derived equivalent of pCN4. Plasmid pHBV DN was co-transfected with "wild type" pHBV into HuH-7 and HepG2 cells. It inhibited "wild type" HBV DNA replication by 90% (Fig. 4).

Fig. 4 shows a Southern blot analysis of core particle associated viral DNA extracted from HepG2 cells five days after transfection. The blot was probed with full length ^{32}P labeled HBV DNA. Lane M contains ^{32}P 5' end labeled lambda HindIII molecular weight markers. Lane 1 contains 3.2 kb linear HBV DNA (10 pg). The remaining lanes show core particle associated viral DNA extracted from cells transfected with pHBV (lane 2); or PHBV and pHBV DN (lane 3).

The constructs pHBV DN AA and pHBV DN BB were assayed in the same manner, for the purpose of mapping which regions of the core protein and of the surface protein were necessary for inhibition. The construct pHBV DN AA was at least as potent an inhibitor as pHBV DN, whereas pHBV DN BB was less inhibitory than pHBV DN. This is shown in Fig. 5, which is a Southern blot analysis illustrating the antiviral effects of the pHBV DN AA and pHBV DN BB dominant negative core mutants on "wild type" HBV replication during transient transfection experiments in HuH-7 cells. The pCMV-HBV lane shows the level of "wild type" HBV replication in HUH-7 cells. The dominant negative mutant pHBV-DN reduced wild type replication by 95%. When this construct was placed in a vector containing the adenovirus sequences necessary for

producing a recombinant adenovirus vector (Ad HBV DN), there was an 80% decrease in HBV replication. When the HBV DN construct was placed in a retroviral vector (e.g., pBP HBV DN), there was a 90-95% reduction in HBV replication.

Experiments were then performed to assess the presence and amount of pregenomic RNA within nucleocapsids, and to compare these results to the level of viral RNA present in the cytosolic fraction by means of a sensitive RNase protection assay (Fig. 6). RNA was extracted from HepG2 transfected cells and probed with a ^{32}P labeled 322 nt RNA probe containing the BamHI (pos. 2906)-EcoRI (pos. 1) fragment (lane P), or electrophoresed on a 6% denaturing PAGE gel after RNase A and T1 digestion. Lane 1 contains 2 μg of total RNA from HepG2 cells transfected with pHBV; lane 2 contains 2 μg of total RNA from HepG2 cells transfected with pHBV and pHBV DN; lane 3 contains 2 μg of total RNA from HepG2 cells transfected with pHBV DN alone (the BamHI-EcoRI fragment is missing in this construct). The remaining lanes show RNA that was extracted from HepG2-derived core particles and then probed as in lanes 1-3. Each lane was loaded with half of the core associated RNA extracted from a 10 cm dish. Lane 4 contains core particle associated RNA from cells transfected with pHBV. Lane 5 contains core particle associated RNA from cells transfected with pHBV and pHBV DN. Lane 6 contains core particle associated RNA from cells transfected with pHBV DN alone. There was a 90% reduction in encapsidation of "wild type" pregenomic RNA when pHBV DN was co-transfected with the wild type HBV DNA expressing plasmid, whereas no significant reduction in viral RNA was observed in experiments performed with total cellular RNA. The riboprobe used in this experiment protects

pregenomic and pre-S1 transcripts, both of which were absent in the pHBV DN transfected cells.

"Wild type" pregenomic viral RNA is incapable of being encapsidated in the presence of mutant core protein because of inefficient core particle assembly. Cell lysates derived from HepG2 cells previously transfected with pHBV alone, pHBV and pHBV DN together, and pHBV DN alone were sedimented on a 20% w/v sucrose cushion for one hour at 500,000 g. Under these experimental conditions non-particulate core protein and HBeAg were left in solution (Zhou et al., *supra*). The pellet was analyzed for core protein by 12.5% SDS-PAGE electrophoresis, and analyzed on a Western blot using polyclonal anti-HBc antibodies as probes (Fig. 7). The viral core particles were derived from: lane 1, cells transfected with pHBV; lane 2, cells transfected with pHBV and pHBV DN; lane 3, cells transfected with pHBV DN alone; lane 4, HepG2 2215 cells (positive control). Lane 5 contains 100 μg of cell lysate in RIPA buffer not subjected to ultracentrifugation and extracted from HepG2 2215 cells to show enrichment of core particles by the pelleting technique (positive control). The protein in lane 6 was derived from the pellets of untransfected HepG2 cells (negative control). A protein band of 21.5 kd, corresponding to the intact "wild type" HBV core protein, was detected only in the pellet derived from HepG2 cells transfected with pHBV. In the pellet of cells transfected with the pHBV DN plasmid, an immunoreactive core protein band of 11.5 kd was detected. This protein was substantially smaller than the predicted size of the full length core-surface fusion protein derived from the pHBV DN (about 38 kd).

To determine whether the HBV core dominant negative mutant HBV DN can make hepatoma cell lines resistant to HBV replication, the HBV DN coding sequence

was cloned into the retroviral vector pBabe Puro (pBP), which contains a puromycin selectable marker. The resulting vector is named pBPHBV DN. Recombinant retroviral stocks were obtained after transfecting pBPHBV DN into the packaging cell line PA317. The stocks were then used to infect HepG2 and HepG2 2215 cell lines. The HepG2 2215 cells constitutively produce wild type HBV virions due to the stable integration of a head to tail dimer of HBV. Pools of stably transduced clones were grown in the presence of puromycin. HBV DNA was purified from the core particles and analyzed by Southern blot. HepG2 2215 transduced by the pBP HBV DN vector showed a 90% reduction in HBV replication when compared to HepG2 2215 cells transduced by the pBP vector (Fig. 8). This result demonstrates a striking reduction of HBV replicative intermediates in core particles, even in a cell line that constitutively expresses all the viral gene products and replicative forms of the virus.

The Flag tagged dominant negative form of the HBV DN sequence was also cloned into the adenoviral vector pAdBglII to generate the vector pAdHBV DN. This vector contains a multiple cloning site flanked by the CMV EI promoter and by adenovirus 5 sequences. The adenovirus 5 sequences allow homologous recombination and reconstitution of a recombinant replication incompetent adenovirus after cotransfection in 293 cells (Graham et al., the Human press, Vol 7, 109-128, 1991). The plasmid pAdHBV DN was then introduced, along with pHBV, into HCC cells by transient transfection, inhibiting HBV replication by 80% (Fig. 5). Adenoviral vectors such as pAd HBV DN can be used to generate a replication incompetent adenovirus by homologous recombination, and can express the HBV DN polypeptide in the liver.

Inhibition of DHBV replication Substantial suppression of DHBV replication was obtained by co-

transfecting pCMV DHBV with the plasmid pBK. The pBK plasmid encodes a DHBV core protein which lacks the last carboxyterminal five amino acids, fused to a surface protein which lacks the aminoterminal first four amino acids. When shorter core fragments were fused in frame to a surface protein lacking the aminoterminal first four amino acids (plasmid pSK), to the Pol gene product (pNX), or to the pre-S gene product (pSK), there was little or no effect on DHBV replication. This result indicated that both the core protein and the surface protein extension were important for exerting an inhibitory effect on "wild type" DHBV replication, presumably by disrupting nucleocapsid assembly. The core portion of the chimeric mutant polypeptide interacts with the wild type core protein, preventing formation of intact nucleocapsids and thus encapsidation of the DHBV pregenome. A construct that expressed only the DHBV core protein (pK) was incapable of inhibiting DHBV replication, while a plasmid that expressed the same core portion as the pBK plasmid but fused to the polymerase gene was incapable of inhibiting "wild type" DHBV replication. Fig. 9 is a Southern Blot analysis of cytosolic derived nucleocapsid DNA from transfected LMC cells, hybridized to a full length DHBV DNA probe. LMC cells were transfected with 10 μg of pCMV DHBV together with 10 μg of mutant plasmids pSK (lane 2), pBK (lane 3), pSK (lane 4, the same as lane 2), pK (lane 5), or pNX (lane 6). The last lane contains the cytosolic derived nucleocapsid DNA from a LMC cell line stably transfected with a head-to-tail DHBV dimer as a positive control. Replication of "wild type" DHBV was inhibited by the dominant negative core mutant construct BK.

Therapeutic Use

The mutant polypeptides of the invention can be provided exogenously to a target cell of an animal suspected of harboring a hepadnavirus infection by any appropriate method, for example by oral, parenteral, transdermal, or transmucosal administration. The mutant polypeptide can be administered in a sustained release formulation using a biodegradable biocompatible polymer, or by on-site delivery using micelles, gels or liposomes. Therapeutic doses can be, but are not necessarily, in the range of 0.01 - 100.0 mg/kg body weight, or a range that is clinically determined to be appropriate by those skilled in the art.

The polypeptides useful in a method of the invention, or as candidate agents in a method of the invention, can be purified using conventional methods of protein isolation known to one skilled in the art. These methods include, but are not limited to, precipitation, chromatography, immunoadsorption, or affinity techniques (see, e.g., Scopes, R. Protein Purification: Principles and Practice, 1982 Springer Verlag, NY). The polypeptide can be purified from starting material that is derived from a genetically engineered cell line. One useful method of purification involves expressing the polypeptide as a fusion protein encoded by a glutathione-S-transferase vector, purifying the resulting fusion protein by GST-GSH affinity chromatography, and removing the GST portion of the fusion polypeptide by thrombin cleavage. Alternatively, a synthetic mutant polypeptide can be prepared by automated peptide synthesis (see, e.g., Ausubel et al., eds. Current Protocols in Molecular Biology, John Wiley & Sons, publ. NY. 1987, 1989; Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual (2d ed.), CSH Press).

Therapeutic administration of a mutant polypeptide can also be accomplished using gene therapy techniques. A nucleic acid that included a promoter operatively linked to a sequence encoding a polypeptide of the invention is used to generate high-level expression of the polypeptide in cells transfected with the nucleic acid. Gene transfer can be performed *ex vivo* or *in vivo*. To administer the nucleic acid *ex vivo*, cells can be removed from the body of the patient, transfected with the nucleic acid encoding the mutant polypeptide, and returned to the patient's body. Alternatively the nucleic acid can be administered *in vivo*, by transfecting the nucleic acid into target cells (e.g., hepatocytes) so that the mutant polypeptide is expressed *in situ*.

The nucleic acid molecule is contained within a non-replicating linear or circular DNA or RNA molecule, or within an autonomously replicating plasmid or viral vector, or may be integrated into the host genome. Any vector that can transfect a cell can be used in the methods of the invention. Preferred vectors are viral vectors, including those derived from replication-defective hepatitis virus (e.g., HBV and HCV), retrovirus (see, e.g., WO89/07136; Rosenberg et al., N. Eng. J. Med. 323(9):570-578, 1990; Miller et al., 1993 *supra*), adenovirus (see, e.g., Morsey et al., J. Cell. Biochem., Supp. 17E, 1993; Graham et al., in Murray, ed., Methods in Molecular Biology: Gene Transfer and Expression Protocols. Vol. 7, Clifton, NJ: the Human Press 1991: 109-128), adeno-associated virus (Kotin et al., Proc. Natl. Acad. Sci. USA 87:2211-2215, 1990), replication defective herpes simplex virus (HSV; Lu et al., Abstract, page 66, Abstracts of the Meeting on Gene Therapy, Sept. 22-26, 1992, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), and any modified versions of these vectors. Other preferred viral vectors include those

modified to target a specific cell type (see, e.g., Kan et al. WO 93/25234; Kasahara et al. Science, 266:1373-76, 1994; Dornburg et al. WO 94/12626; Russell et al. WO 94/06920). Methods for constructing expression vectors are well known in the art (see, e.g., Molecular Cloning: A Laboratory Manual, Sambrook et al., eds., Cold Spring Harbor Laboratory, 2nd Edition, Cold Spring Harbor, New York, 1989).

Appropriate regulatory sequences can be inserted into the vectors of the invention using methods known to those skilled in the art, e.g., by homologous recombination (Graham et al., *J. Gen. Virol.* 36:59-72, 1977), or by other appropriate methods (Sambrook et al., eds., *supra*). Promoters are inserted into the vectors so that they are operatively linked 5' to the nucleic acid sequence encoding the mutant polypeptide. Any promoter that is able to initiate transcription in a target cell can be used in the invention. For example, non-tissue specific promoters, such as the cytomegalovirus (DeBernardi et al., Proc. Natl. Acad. Sci. USA 88:9257-9261, 1991, and references therein), mouse metallothionine I gene (Hammer, et al., J. Mol. Appl. Gen. 1:273-288, 1982), HSV thymidine kinase (McKnight, Cell, 31:355-365, 1982), and SV40 early (Benoist et al., Nature, 290:304-310, 1981) promoters may be used. Preferred promoters for use in the invention are hepatocyte-specific promoters, the use of which ensures that the mutant polypeptides are expressed primarily in hepatocytes. Preferred hepatocyte-specific promoters include, but are not limited to the albumin, alpha-fetoprotein, alpha-1-antitrypsin, retinol-binding protein, and asialoglycoprotein receptor promoters. Additional viral promoters and enhancers, such as those from herpes simplex virus (types I and II), hepatitis virus (Types A, B, and C), and Rous sarcoma virus (RSV;

Fang et al., Hepatology 10:781-787, 1989), can also be used in the invention.

The mutant polypeptides of the invention, and the recombinant vectors containing nucleic acid sequences encoding them, may be used in therapeutic compositions for preventing or treating HBV infection. The therapeutic compositions of the invention may be used alone or in admixture, or in chemical combination, with one or more materials, including other mutant polypeptides or recombinant vectors, materials that increase the biological stability of the oligonucleotides or the recombinant vectors, or materials that increase the ability of the therapeutic compositions to penetrate hepatocytes selectively. The therapeutic compositions of the invention can be administered in pharmaceutically acceptable carriers (e.g., physiological saline), which are selected on the basis of the mode and route of administration, and standard pharmaceutical practice. Suitable pharmaceutical carriers, as well as pharmaceutical necessities for use in pharmaceutical formulations, are described in *Remington's Pharmaceutical Sciences*, a standard reference text in this field.

The therapeutic compositions of the invention can be administered in dosages determined to be appropriate by one skilled in the art. An appropriate dosage is one which effects a reduction in a disease caused by HBV infection. It is expected that the dosages will vary, depending upon the pharmacokinetic and pharmacodynamic characteristics of the particular agent, and its mode and route of administration, as well as the age, weight, and health (including renal and hepatic function) of the recipient; the nature and extent of the disease; the frequency and duration of the treatment; the type of, if any, concurrent therapy; and the desired effect. It is expected that a useful dosage contains between about 0.1

to 100 mg of active ingredient per kilogram of body weight. Ordinarily a dosage of 0.5 to 50 mg, and preferably, 1 to 10 mg of active ingredient per kilogram of body weight per day given in divided doses, or in sustained release form, is appropriate.

The therapeutic compositions of the invention may be administered to a patient by any appropriate mode, e.g., parenterally, as determined by one skilled in the art. Alternatively, it may by necessary to administer the treatment surgically to the target tissue. The treatments of the invention may be repeated as needed, as determined by one skilled in the art.

The invention also includes any other methods which accomplish *in vivo* transfer of nucleic acids into target cells. For example, the nucleic acids may be packaged into liposomes, non-viral nucleic acid-based vectors, erythrocyte ghosts, or microspheres (microparticles; see, e.g., U.S. Patent No. 4,789,734; U.S. Patent No. 4,925,673; U.S. Patent No. 3,625,214; Gregoriadis, Drug Carriers in Biology and Medicine, pp. 287-341 (Academic Press, 1979)). Further, delivery of mutant polypeptides be accomplished by direct injection of their nucleic acid coding sequences into target tissues, for example, in a calcium phosphate precipitate or coupled with lipids, or as "naked DNA".

Mutant core polypeptides and core-surface fusion proteins of the invention can be tested for their ability to inhibit hepadnavirus replication in an animal model. For example, candidate polypeptides can be injected into an animal that is infected with a hepadnavirus, e.g., a woodchuck, duck, or ground squirrel harboring its respective hepatitis B virus variants (see, e.g., Mason et al., *J. Virol*. 36:829, 1980; Schodel et al., in Molecular Biology of hepatitis B virus, CRC press, Boca Raton, p. 53-80, 1991; Summers et al., *Proc. Natl. Acad.*

17:39:34

Sci. USA, 75:4533-4537, 1978). Candidate polypeptides can also be analyzed in transgenic animal strains developed for the purpose of studying hepadnaviral gene expression (see, e.g., Babinet et al., Science, 230:1160-63, 1985; Burk et al., J. Virol. 62:649-54, 1988; Chisari et al., Science 230:1157-60, 1985; Chisari, in Current Topics in Microbiology and Immunology, p. 85-101, 1991). Candidate polypeptides of the invention can also be tested in animals that are naturally infected with HBV, e.g., in chimpanzees, by administering the polypeptides, or the nucleic acids encoding them, to the animal by one of the methods discussed above, or by other standard methods.

Other Embodiments

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

All publications cited herein are fully incorporated by reference in their entirety.

Other embodiments are within the claims set forth below.

TABLE 1

CONSERVATIVE AMINO ACID REPLACEMENTS

For Amino Acid	Code	Replace With
Alanine	A	D-Ala, Gly, Aib, β-Ala, Acp, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, Aib, β-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, AdaA, AdaG, cis-3,4, or 5-phenylproline, Bpa, D-Bpa
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D-or L-1-oxazolidine-4-carboxylic acid (Kauer, U.S. Patent (4,511,390)
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met, AdaA, AdaG

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The General Hospital Corporation

(ii) TITLE OF INVENTION: INHIBITION OF HEPATITIS B REPLICATION

(iii) NUMBER OF SEQUENCES: 14

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson P.C.
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/017,814
(B) FILING DATE: 20-JUN-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Clark, Paul T.
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 00786/282001

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617/542-5070
(B) TELEFAX: 617/542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1041 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG GAC ATA GAT CCC TAT AAA GAA TTT GGT TCA TCT TAT CAG TTG TTG       48
Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ser Ser Tyr Gln Leu Leu
  1               5                  10                  15

AAT TTT CTT CCT TTG GAC TTC TTT CCT GAC CTT AAT GCT TTG GTG GAC       96
Asn Phe Leu Pro Leu Asp Phe Phe Pro Asp Leu Asn Ala Leu Val Asp
            20                  25                  30
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ACT GCT ACT GCC TTG TAT GAA GAA GAG CTA ACA GGT AGG GAA CAT TGC      144
Thr Ala Thr Ala Leu Tyr Glu Glu Glu Leu Thr Gly Arg Glu His Cys
         35                  40                  45

TCT CCG CAC CAT ACA GCT ATT AGA CAA GCT TTA GTA TGC TGG GAT GAA      192
Ser Pro His His Thr Ala Ile Arg Gln Ala Leu Val Cys Trp Asp Glu
     50                  55                  60

TTA ACT AAA TTG ATA GCT TGG ATG AGC TCT AAC ATA ACT TCT GAA CAA      240
Leu Thr Lys Leu Ile Ala Trp Met Ser Ser Asn Ile Thr Ser Glu Gln
 65                  70                  75                  80

GTA AGA ACA ATC ATA GTA AAT CAT GTC AAT GAT ACC TGG GGA CTT AAG      288
Val Arg Thr Ile Ile Val Asn His Val Asn Asp Thr Trp Gly Leu Lys
                 85                  90                  95

GTG AGA CAA AGT TTA TGG TTT CAT TTG TCA TGT CTC ACT TTC GGA CAA      336
Val Arg Gln Ser Leu Trp Phe His Leu Ser Cys Leu Thr Phe Gly Gln
            100                 105                 110

CAT ACA GTT CAA GAA TTT TTA GTA AGT TTT GTA GTA TGG ATC AGA ACT      384
His Thr Val Gln Glu Phe Leu Val Ser Phe Val Val Trp Ile Arg Thr
        115                 120                 125

CCA GCT CCA TAT AGA CCT CCT AAT GCA CCC ATT CTC TCG ACT CTT CCG      432
Pro Ala Pro Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
    130                 135                 140

GAA CAT ACA GTC ATT AGA AGA GGA GGT GCA AGA GCT TCT AGG TCC CCC      480
Glu His Thr Val Ile Arg Arg Gly Gly Ala Arg Ala Ser Arg Ser Pro
145                 150                 155                 160

AGA AGA CGC ACT CCC TCT CCT CGC AGG AGA AGA TCC CAA AAT TCG CAG      528
Arg Arg Arg Thr Pro Ser Pro Arg Arg Arg Arg Ser Gln Asn Ser Gln
                165                 170                 175

TTC CAA ACT TGC AAA CAC TTG CCA ACC TCC TGT CCA CCA ACT TGC AAT      576
Phe Gln Thr Cys Lys His Leu Pro Thr Ser Cys Pro Pro Thr Cys Asn
            180                 185                 190

GGC TTT CGT TGG ATG TAT CTG CGG CGT TTT ATC ATA TAC CTA TTA GTC      624
Gly Phe Arg Trp Met Tyr Leu Arg Arg Phe Ile Ile Tyr Leu Leu Val
        195                 200                 205

CTG CTG CTG TGC CTC ATC TTC TTG TTG GTT CTC CTG GAC TGG AAA GGT      672
Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu Asp Trp Lys Gly
    210                 215                 220

TTA ATA CCT GTC TGT CCT CTT CAA CCC ACA ACA GAA ACA ACA GTC AAT      720
Leu Ile Pro Val Cys Pro Leu Gln Pro Thr Thr Glu Thr Thr Val Asn
225                 230                 235                 240

TGC AGA CAA TGC ACA ATC TCT GCA CAA GAC ATG TAT ACT CCT CCT TAC      768
Cys Arg Gln Cys Thr Ile Ser Ala Gln Asp Met Tyr Thr Pro Pro Tyr
                245                 250                 255

TGT TGT TGT TTA AAA CCT ACG GCA GGA AAT TGC ACT TGT TGG CCC ATC      816
Cys Cys Cys Leu Lys Pro Thr Ala Gly Asn Cys Thr Cys Trp Pro Ile
            260                 265                 270

CCT TCA TCA TGG GCT TTA GGA AAT TAC CTA TGG GAG TGG GCC TTA GCT      864
Pro Ser Ser Trp Ala Leu Gly Asn Tyr Leu Trp Glu Trp Ala Leu Ala
        275                 280                 285
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CGT CTC TCT TGG CTC AAT TTA CTA GTG CCC TTG CTT CAA TGG TTA GGA      912
Arg Leu Ser Trp Leu Asn Leu Leu Val Pro Leu Leu Gln Trp Leu Gly
    290                 295                 300

GGA ATT TCC CTC ATT GCG TGG TTT TTG CTT ATA TGG ATG ATT TGG TTT      960
Gly Ile Ser Leu Ile Ala Trp Phe Leu Leu Ile Trp Met Ile Trp Phe
305                 310                 315                 320

TGG GGG CCC GCA CTT CTG AGC ATC TTA CCG CCA TTT ATT CCC ATA TTT     1008
Trp Gly Pro Ala Leu Leu Ser Ile Leu Pro Pro Phe Ile Pro Ile Phe
                325                 330                 335

GTT CTG TTT TTC TTG ATT TGG GTA TAC ATT  T GA                       1041
Val Leu Phe Phe Leu Ile Trp Val Tyr Ile
            340                 345
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 346 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ser Ser Tyr Gln Leu Leu
  1               5                  10                  15

Asn Phe Leu Pro Leu Asp Phe Phe Pro Asp Leu Asn Ala Leu Val Asp
             20                  25                  30

Thr Ala Thr Ala Leu Tyr Glu Glu Glu Leu Thr Gly Arg Glu His Cys
         35                  40                  45

Ser Pro His His Thr Ala Ile Arg Gln Ala Leu Val Cys Trp Asp Glu
     50                  55                  60

Leu Thr Lys Leu Ile Ala Trp Met Ser Ser Asn Ile Thr Ser Glu Gln
 65                  70                  75                  80

Val Arg Thr Ile Ile Val Asn His Val Asn Asp Thr Trp Gly Leu Lys
                 85                  90                  95

Val Arg Gln Ser Leu Trp Phe His Leu Ser Cys Leu Thr Phe Gly Gln
            100                 105                 110

His Thr Val Gln Glu Phe Leu Val Ser Phe Val Val Trp Ile Arg Thr
        115                 120                 125

Pro Ala Pro Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
    130                 135                 140

Glu His Thr Val Ile Arg Arg Gly Gly Ala Arg Ala Ser Arg Ser Pro
145                 150                 155                 160

Arg Arg Arg Thr Pro Ser Pro Arg Arg Arg Arg Ser Gln Asn Ser Gln
                165                 170                 175

Phe Gln Thr Cys Lys His Leu Pro Thr Ser Cys Pro Pro Thr Cys Asn
            180                 185                 190
```

Gly Phe Arg Trp Met Tyr Leu Arg Arg Phe Ile Ile Tyr Leu Leu Val
195 200 205

Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu Asp Trp Lys Gly
210 215 220

Leu Ile Pro Val Cys Pro Leu Gln Pro Thr Thr Glu Thr Thr Val Asn
225 230 235 240

Cys Arg Gln Cys Thr Ile Ser Ala Gln Asp Met Tyr Thr Pro Pro Tyr
245 250 255

Cys Cys Cys Leu Lys Pro Thr Ala Gly Asn Cys Thr Cys Trp Pro Ile
260 265 270

Pro Ser Ser Trp Ala Leu Gly Asn Tyr Leu Trp Glu Trp Ala Leu Ala
275 280 285

Arg Leu Ser Trp Leu Asn Leu Leu Val Pro Leu Leu Gln Trp Leu Gly
290 295 300

Gly Ile Ser Leu Ile Ala Trp Phe Leu Leu Ile Trp Met Ile Trp Phe
305 310 315 320

Trp Gly Pro Ala Leu Leu Ser Ile Leu Pro Pro Phe Ile Pro Ile Phe
325 330 335

Val Leu Phe Phe Leu Ile Trp Val Tyr Ile
340 345

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1056 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCT CGC AGG ATG GAC ATC GAC CCT TAT AAA GAA TTT GGA GCT ACT GTG 48
Pro Arg Arg Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val
350 355 360

GAG TTA CTC TCG TTT TTG CCT TCT GAC TTC TTT CCT TCA GTA CGA GAT 96
Glu Leu Leu Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Arg Asp
365 370 375

CTT CTA GAT ACC GCC TCA GCT CTG TAT CGG GAA GCC TTA GAG TCT CCT 144
Leu Leu Asp Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro
380 385 390

GAG CAT TGT TCA CCT CAC CAT ACT GCA CTC AGG CAA GCA ATT CTT TGC 192
Glu His Cys Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys
395 400 405 410

TGG GGG GAA CTA ATG ACT CTA GCT ACC TGG GTG GGT GTT AAT TTG GAA 240
Trp Gly Glu Leu Met Thr Leu Ala Thr Trp Val Gly Val Asn Leu Glu
415 420 425

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GAT CCA GCG TCT AGA GAC CTA GTA GTC AGT TAT GTC AAC ACT AAT ATG      288
Asp Pro Ala Ser Arg Asp Leu Val Val Ser Tyr Val Asn Thr Asn Met
            430                 435                 440

GGC CTA AAG TTC AGG CAA CTC TTG TGG TTT CAC ATT TCT TGT CTC ACT      336
Gly Leu Lys Phe Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr
        445                 450                 455

TTT GGA AGA GAA ACA GTT ATA GAG TAT TTG GTG TCT TTC GGA GTG TGG      384
Phe Gly Arg Glu Thr Val Ile Glu Tyr Leu Val Ser Phe Gly Val Trp
    460                 465                 470

ATT CGC ACT CCT CCA GCT TAT AGA CCA CCA AAT GCC CCT ATC CTA TCA      432
Ile Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser
475                 480                 485                 490

ACA CTT CCG GAA CAT ACA GTC ATT AGA AGA GGA GGT GCA AGA GCT TCT      480
Thr Leu Pro Glu His Thr Val Ile Arg Arg Gly Gly Ala Arg Ala Ser
                495                 500                 505

AGG TCC CCC AGA AGA CGC ACT CCC TCT CCT CGC AGG AGA AGA TCC CAA      528
Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro Arg Arg Arg Arg Ser Gln
            510                 515                 520

AAT TCG CAG TCC CCA ACC TCC AAT CAC TCA CCA ACC TCT TGT CCT CCA      576
Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys Pro Pro
        525                 530                 535

ACT TGT CCT GGT TAT CGC TGG ATG TGT CTG CGG CGT TTT ATC ATC TTC      624
Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile Phe
    540                 545                 550

CTC TTC ATC CTG CTG CTA TGC CTC ATC TTC TTG TTG GTT CTT CTG GAC      672
Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu Asp
555                 560                 565                 570

TAT CAA GGT ATG TTG CCC GTT TGT CCT CTA ATT CCA GGA TCC TCA ACA      720
Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Ser Thr
                575                 580                 585

ACC AGC ACG GGA CCA TGC CGG ACC TGC ATG ACT ACT GCT CAA GGA ACC      768
Thr Ser Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala Gln Gly Thr
            590                 595                 600

TCT ATG TAT CCC TCC TGT TGC TGT ACC AAA CCT TCG GAC GGA AAT TGC      816
Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly Asn Cys
        605                 610                 615

ACC TGT ATT CCC ATC CCA TCA TCC TGG GCT TTC GGA AAA TTC CTA TGG      864
Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe Leu Trp
    620                 625                 630

GAG TGG GCC TCA GCC CGT TTC TCC TGG CTC AGT TTA CTA GTG CCA TTT      912
Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val Pro Phe
635                 640                 645                 650

GTT CAG TGG TTC GTA GGG CTT TCC CCC ACT GTT TGG CTT TCA GTT ATA      960
Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val Ile
                655                 660                 665

TGG ATG ATG TGG TAT TGG GGG CCA AGT CTG TAC AGC ATC TTG AGT CCC     1008
Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile Leu Ser Pro
            670                 675                 680
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TTT TTA CCG CTG TTA CCA ATT TTC TTT TGT CTT TGG GTA TAC ATT T 1054
Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
685 690 695

AA 1056

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 351 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Pro Arg Arg Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val
1 5 10 15

Glu Leu Leu Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Arg Asp
20 25 30

Leu Leu Asp Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro
35 40 45

Glu His Cys Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys
50 55 60

Trp Gly Glu Leu Met Thr Leu Ala Thr Trp Val Gly Val Asn Leu Glu
65 70 75 80

Asp Pro Ala Ser Arg Asp Leu Val Val Ser Tyr Val Asn Thr Asn Met
85 90 95

Gly Leu Lys Phe Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr
100 105 110

Phe Gly Arg Glu Thr Val Ile Glu Tyr Leu Val Ser Phe Gly Val Trp
115 120 125

Ile Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser
130 135 140

Thr Leu Pro Glu His Thr Val Ile Arg Arg Gly Gly Ala Arg Ala Ser
145 150 155 160

Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro Arg Arg Arg Arg Ser Gln
165 170 175

Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys Pro Pro
180 185 190

Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile Phe
195 200 205

Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu Asp
210 215 220

Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Ser Thr
225 230 235 240

Thr Ser Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala Gln Gly Thr
245 250 255

Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly Asn Cys
260 265 270

Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe Leu Trp
275 280 285

Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val Pro Phe
290 295 300

Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val Ile
305 310 315 320

Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile Leu Ser Pro
325 330 335

Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
340 345 350

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1194 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATG GAC ATC GAC CCT TAT AAA GAA TTT GGA GCT ACT GTG GAG TTA CTC 48
Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu
355 360 365

TCG TTT TTG CCT TCT GAC TTC TTT CCT TCA GTA CGA GAT CTT CTA GAT 96
Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp
370 375 380

ACC GCC TCA GCT CTG TAT CGG GAA GCC TTA GAG TCT CCT GAG CAT TGT 144
Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
385 390 395

TCA CCT CAC CAT ACT GCA CTC AGG CAA GCA ATT CTT TGC TGG GGG GAA 192
Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
400 405 410 415

CTA ATG ACT CTA GCT ACC TGG GTG GGT GTT AAT TTG GAA GAT CCA GCG 240
Leu Met Thr Leu Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala
420 425 430

TCT AGA GAC CTA GTA GTC AGT TAT GTC AAC ACT AAT ATG GGC CTA AAG 288
Ser Arg Asp Leu Val Val Ser Tyr Val Asn Thr Asn Met Gly Leu Lys
435 440 445

TTC AGG CAA CTC TTG TGG TTT CAC ATT TCT TGT CTC ACT TTT GGA AGA 336
Phe Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg
450 455 460

GAA ACA GTT ATA GAG TAT TTG GTG TCT TTC GGA GTG TGG ATT CGC ACT 384
Glu Thr Val Ile Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
465 470 475

CCT CCA GCT TAT AGA CCA CCA AAT GCC CCT ATC CTA TCA ACA CTT CCG 432
Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
480 485 490 495

GAG ACT ACT GTT GTT AGA CGA CGA GGC AGG TCC CCT AGA AGA AGA ACT 480
Glu Thr Thr Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr
500 505 510

CCC TCG CCT CGC AGA CGA AGG TCT CAA TCG CCG CGT CGC AGA AGA TCT 528
Pro Ser Pro Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser
515 520 525

CAA TCT CGG CTA GGA CCC CTT CTC GTG TTA CAG GCG GGG TTT TTC TTG 576
Gln Ser Arg Leu Gly Pro Leu Leu Val Leu Gln Ala Gly Phe Phe Leu
530 535 540

TTG ACA AGA ATC CTC ACA ATA CCG CAG AGT CTA GAC TCG TGG TGG ACT 624
Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp Ser Trp Trp Thr
545 550 555

TCT CTC AAT TTT CTA GGG GGA ACT ACC GTG TGT CTT GGC CAA AAT TCG 672
Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu Gly Gln Asn Ser
560 565 570 575

CAG TCC CCA ACC TCC AAT CAC TCA CCA ACC TCT TGT CCT CCA ACT TGT 720
Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys Pro Pro Thr Cys
580 585 590

CCT GGT TAT CGC TGG ATG TGT CTG CGG CGT TTT ATC ATC TTC CTC TTC 768
Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe
595 600 605

ATC CTG CTG CTA TGC CTC ATC TTC TTG TTG GTT CTT CTG GAC TAT CAA 816
Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln
610 615 620

GGT ATG TTG CCC GTT TGT CCT CTA ATT CCA GGA TCC TCA ACA ACC AGC 864
Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Ser Thr Thr Ser
625 630 635

ACG GGA CCA TGC CGG ACC TGC ATG ACT ACT GCT CAA GGA ACC TCT ATG 912
Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala Gln Gly Thr Ser Met
640 645 650 655

TAT CCC TCC TGT TGC TGT ACC AAA CCT TCG GAC GGA AAT TGC ACC TGT 960
Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly Asn Cys Thr Cys
660 665 670

ATT CCC ATC CCA TCA TCC TGG GCT TTC GGA AAA TTC CTA TGG GAG TGG 1008
Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe Leu Trp Glu Trp
675 680 685

GCC TCA GCC CGT TTC TCC TGG CTC AGT TTA CTA GTG CCA TTT GTT CAG 1056
Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val Pro Phe Val Gln
690 695 700

TGG TTC GTA GGG CTT TCC CCC ACT GTT TGG CTT TCA GTT ATA TGG ATG 1104
Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val Ile Trp Met
705 710 715

ATG TGG TAT TGG GGG CCA AGT CTG TAC AGC ATC TTG AGT CCC TTT TTA 1152
Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile Leu Ser Pro Phe Leu
720 725 730 735

CCG CTG TTA CCA ATT TTC TTT TGT CTT TGG GTA TAC ATT T AA 1194
Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
740 745

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 397 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu
1 5 10 15

Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp
20 25 30

Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
35 40 45

Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
50 55 60

Leu Met Thr Leu Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala
65 70 75 80

Ser Arg Asp Leu Val Val Ser Tyr Val Asn Thr Asn Met Gly Leu Lys
85 90 95

Phe Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg
100 105 110

Glu Thr Val Ile Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
115 120 125

Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
130 135 140

Glu Thr Thr Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr
145 150 155 160

Pro Ser Pro Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser
165 170 175

Gln Ser Arg Leu Gly Pro Leu Leu Val Leu Gln Ala Gly Phe Phe Leu
180 185 190

Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp Ser Trp Trp Thr
195 200 205

Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu Gly Gln Asn Ser
210 215 220

Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys Pro Pro Thr Cys
225 230 235 240

Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe
245 250 255

Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln
260 265 270

Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Ser Thr Thr Ser
275 280 285

Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala Gln Gly Thr Ser Met
290 295 300

Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly Asn Cys Thr Cys
305 310 315 320

Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe Leu Trp Glu Trp
325 330 335

Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val Pro Phe Val Gln
340 345 350

Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val Ile Trp Met
355 360 365

Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile Leu Ser Pro Phe Leu
370 375 380

Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
385 390 395

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 870 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG GAC ATC GAC CCT TAT AAA GAA TTT GGA GCT ACT GTG GAG TTA CTC 48
Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu
400 405 410

TCG TTT TTG CCT TCT GAC TTC TTT CCT TCA GTA CGA GAT CTT CTA GAT 96
Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp
415 420 425

ACC GCC TCA GCT CTG TAT CGG GAA GCC TTA GAG TCT CCT GAG CAT TGT 144
Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
430 435 440 445

TCA CCT CAC CAT ACT GCA CTC AGG CAA GCA ATT CTT TGC TGG GGG GAA 192
Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
450 455 460

```
CTA ATG ACT CTA GCT ACC TGG GTG GGT GTT AAT TTG GAA GAT CCA GCG     240
Leu Met Thr Leu Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala
            465                 470                 475

TCT AGA GAC CTA GTA GTC AGT TAT GTC AAC ACT AAT ATG GGC CTA AAG     288
Ser Arg Asp Leu Val Val Ser Tyr Val Asn Thr Asn Met Gly Leu Lys
        480                 485                 490

TTC AGG CAA CTC TTG TGG TTT CAC ATT TCT TGT CTC ACT TTT GGA AGA     336
Phe Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg
    495                 500                 505

GAA ACA GTT ATA GAG TAT TTG GTG TCT TTC GGA GTG TGG ATT CGC ACT     384
Glu Thr Val Ile Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
510                 515                 520                 525

CCT CCA GCT TAT AGA CCA CCA AAT GCC CCT ATC CTA TCA ACA CTT CCG     432
Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
                530                 535                 540

GAG ACT ACT GTT GTT AGA CGA CGA GGC AGG TCC CCT AGA AGA AGA ACT     480
Glu Thr Thr Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr
            545                 550                 555

CCC TCG CCT CGC AGA CGA AGG TCT CAA TCG CCG CGT CGC AGA AGA TCC     528
Pro Ser Pro Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser
        560                 565                 570

TCA ACA ACC AGC ACG GGA CCA TGC CGG ACC TGC ATG ACT ACT GCT CAA     576
Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala Gln
    575                 580                 585

GGA ACC TCT ATG TAT CCC TCC TGT TGC TGT ACC AAA CCT TCG GAC GGA     624
Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly
590                 595                 600                 605

AAT TGC ACC TGT ATT CCC ATC CCA TCA TCC TGG GCT TTC GGA AAA TTC     672
Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe
                610                 615                 620

CTA TGG GAG TGG GCC TCA GCC CGT TTC TCC TGG CTC AGT TTA CTA GTG     720
Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val
            625                 630                 635

CCA TTT GTT CAG TGG TTC GTA GGG CTT TCC CCC ACT GTT TGG CTT TCA     768
Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser
        640                 645                 650

GTT ATA TGG ATG ATG TGG TAT TGG GGG CCA AGT CTG TAC AGC ATC TTG     816
Val Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile Leu
    655                 660                 665

AGT CCC TTT TTA CCG CTG TTA CCA ATT TTC TTT TGT CTT TGG GTA TAC     864
Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr
670                 675                 680                 685

ATT  T AA                                                           870
Ile
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 289 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu
 1               5                  10                  15

Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp
            20                  25                  30

Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
        35                  40                  45

Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
    50                  55                  60

Leu Met Thr Leu Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala
65                  70                  75                      80

Ser Arg Asp Leu Val Val Ser Tyr Val Asn Thr Asn Met Gly Leu Lys
                85                  90                  95

Phe Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg
            100                 105                 110

Glu Thr Val Ile Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
        115                 120                 125

Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
    130                 135                 140

Glu Thr Thr Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr
145                 150                 155                     160

Pro Ser Pro Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser
                165                 170                 175

Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala Gln
            180                 185                 190

Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly
        195                 200                 205

Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe
    210                 215                 220

Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val
225                 230                 235                     240

Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser
                245                 250                 255

Val Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile Leu
            260                 265                 270

Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr
        275                 280                 285
```

Ile

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1263 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
ATG GAT ATC AAT GCT TCT AGA GCC TTA GCC AAT GTG TAT GAT CTA CCA      48
Met Asp Ile Asn Ala Ser Arg Ala Leu Ala Asn Val Tyr Asp Leu Pro
290                 295                 300                 305

GAT GAT TTC TTT CCA AAA ATA GAT GAT CTT GTT AGA GAT GCT AAA GAC      96
Asp Asp Phe Phe Pro Lys Ile Asp Asp Leu Val Arg Asp Ala Lys Asp
                310                 315                 320

GCT TTA GAG CCT TAT TGG AAA TCA GAT TCA ATA AAG AAA CAT GTT TTG     144
Ala Leu Glu Pro Tyr Trp Lys Ser Asp Ser Ile Lys Lys His Val Leu
            325                 330                 335

ATT GCA ACT CAC TTT GTG GAT CTT ATT GAA GAC TTC TGG CAG ACT ACA     192
Ile Ala Thr His Phe Val Asp Leu Ile Glu Asp Phe Trp Gln Thr Thr
        340                 345                 350

CAG GGC ATG CAT GAA ATA GCC GAA TCA TTA AGA GCT GTT ATA CCT CCC     240
Gln Gly Met His Glu Ile Ala Glu Ser Leu Arg Ala Val Ile Pro Pro
    355                 360                 365

ACT ACT ACT CCT GTT CCA CCG GGT TAT CTT ATT CAG CAC GAA GAA GCT     288
Thr Thr Thr Pro Val Pro Pro Gly Tyr Leu Ile Gln His Glu Glu Ala
370                 375                 380                 385

GAA GAG ATA CCT TTG GGA GAT TTA TTT AAA CAC CAA GAA GAA AGG ATA     336
Glu Glu Ile Pro Leu Gly Asp Leu Phe Lys His Gln Glu Glu Arg Ile
                390                 395                 400

GTG AGT TTC CAA CCC GAC TAT CCG ATT ACG GCT AGA ATT CAT GCT CAT     384
Val Ser Phe Gln Pro Asp Tyr Pro Ile Thr Ala Arg Ile His Ala His
            405                 410                 415

TTG AAA GCT TAT GCA AAA ATT AAC GAG GAA TCA CTG GAT AGG GCT AGG     432
Leu Lys Ala Tyr Ala Lys Ile Asn Glu Glu Ser Leu Asp Arg Ala Arg
        420                 425                 430

AGA TTG CTT TGG TGG CAT TAC AAC TGT TTA CTG TGG GGA GAA GCT CAA     480
Arg Leu Leu Trp Trp His Tyr Asn Cys Leu Leu Trp Gly Glu Ala Gln
    435                 440                 445

GTT ACT AAC TAT ATT TCT CGC TTG CGT ACT TGG TTG TCA ACT CCT GAG     528
Val Thr Asn Tyr Ile Ser Arg Leu Arg Thr Trp Leu Ser Thr Pro Glu
450                 455                 460                 465
```

AAA TAT AGA GGT AGA GAT GCC CCG ACC ATT GAA GCA ATC ACT AGA CCA 576
Lys Tyr Arg Gly Arg Asp Ala Pro Thr Ile Glu Ala Ile Thr Arg Pro
470 475 480

ATC CAG GTG GCT CAG GGA GGC CGA AAA ACA ACT ACG GGT ACT AGA AAA 624
Ile Gln Val Ala Gln Gly Gly Arg Lys Thr Thr Thr Gly Thr Arg Lys
485 490 495

CCT CGT GGA CTC GAA CCT AGA AGA AGA AAA GTT AAA ACC ACA GTT GTC 672
Pro Arg Gly Leu Glu Pro Arg Arg Arg Lys Val Lys Thr Thr Val Val
500 505 510

TAT GGG AGA AGA CGT TCA AAG TCC CGG GGA AGG AGA GCC CCT ACA CCC 720
Tyr Gly Arg Arg Arg Ser Lys Ser Arg Gly Arg Arg Ala Pro Thr Pro
515 520 525

CAA CGT GCG GGC TCC CCT CTC CCA CGT AGT TCG AGC AGC CAC CAT AGA 768
Gln Arg Ala Gly Ser Pro Leu Pro Arg Ser Ser Ser Ser His His Arg
530 535 540 545

TCC TTC GGG GGA ATA CTA GCT GGC CTA ATC GGA TTA CTG GTA AGC TTT 816
Ser Phe Gly Gly Ile Leu Ala Gly Leu Ile Gly Leu Leu Val Ser Phe
550 555 560

TTC TTG TTG ATA AAA ATT CTA GAA ATA CTG AGG AGG CTA GAT TGG TGG 864
Phe Leu Leu Ile Lys Ile Leu Glu Ile Leu Arg Arg Leu Asp Trp Trp
565 570 575

TGG ATT TCT CTC AGT TCT CCA AAG GGA AAA ATG CAA TGC GCT TTC CAA 912
Trp Ile Ser Leu Ser Ser Pro Lys Gly Lys Met Gln Cys Ala Phe Gln
580 585 590

GAT ACT GGA GCC CAA ATC TCT CCA CAT TAC GTC GGA TCT TGC CCG TGG 960
Asp Thr Gly Ala Gln Ile Ser Pro His Tyr Val Gly Ser Cys Pro Trp
595 600 605

GGA TGC CCA GGA TTT CTT TGG ACC TAT CTC AGG CTT TTT ATC ATC TTC 1008
Gly Cys Pro Gly Phe Leu Trp Thr Tyr Leu Arg Leu Phe Ile Ile Phe
610 615 620 625

CTC TTA ATC CTG CTA GTA GCA GCA GGC TTG CTG TAT CTG ACG GAC AAC 1056
Leu Leu Ile Leu Leu Val Ala Ala Gly Leu Leu Tyr Leu Thr Asp Asn
630 635 640

GGG TCT ACT ATT TTA GGA AAG CTC CAA TGG GCG TCG GTC TCA GCC CTT 1104
Gly Ser Thr Ile Leu Gly Lys Leu Gln Trp Ala Ser Val Ser Ala Leu
645 650 655

TTC TCC TCC ATC TCT TCA CTA CTG CCC TCG GAT CCG AAA TCT CTC GTC 1152
Phe Ser Ser Ile Ser Ser Leu Leu Pro Ser Asp Pro Lys Ser Leu Val
660 665 670

GCT TTA ACG TTT GGA CTT TCA CTT ATA TGG ATG ACT TCC TCC TCT GCC 1200
Ala Leu Thr Phe Gly Leu Ser Leu Ile Trp Met Thr Ser Ser Ser Ala
675 680 685

ACC CAA ACG CTC GTC ACC TTA ACG CAA TTA GCC ACG CTG TCT GCT CTT 1248
Thr Gln Thr Leu Val Thr Leu Thr Gln Leu Ala Thr Leu Ser Ala Leu
690 695 700 705

TTT TAC AAG AGC T AG 1263
Phe Tyr Lys Ser

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 420 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Asp Ile Asn Ala Ser Arg Ala Leu Ala Asn Val Tyr Asp Leu Pro
1 5 10 15

Asp Asp Phe Phe Pro Lys Ile Asp Asp Leu Val Arg Asp Ala Lys Asp
20 25 30

Ala Leu Glu Pro Tyr Trp Lys Ser Asp Ser Ile Lys Lys His Val Leu
35 40 45

Ile Ala Thr His Phe Val Asp Leu Ile Glu Asp Phe Trp Gln Thr Thr
50 55 60

Gln Gly Met His Glu Ile Ala Glu Ser Leu Arg Ala Val Ile Pro Pro
65 70 75 80

Thr Thr Thr Pro Val Pro Pro Gly Tyr Leu Ile Gln His Glu Glu Ala
85 90 95

Glu Glu Ile Pro Leu Gly Asp Leu Phe Lys His Gln Glu Glu Arg Ile
100 105 110

Val Ser Phe Gln Pro Asp Tyr Pro Ile Thr Ala Arg Ile His Ala His
115 120 125

Leu Lys Ala Tyr Ala Lys Ile Asn Glu Glu Ser Leu Asp Arg Ala Arg
130 135 140

Arg Leu Leu Trp Trp His Tyr Asn Cys Leu Leu Trp Gly Glu Ala Gln
145 150 155 160

Val Thr Asn Tyr Ile Ser Arg Leu Arg Thr Trp Leu Ser Thr Pro Glu
165 170 175

Lys Tyr Arg Gly Arg Asp Ala Pro Thr Ile Glu Ala Ile Thr Arg Pro
180 185 190

Ile Gln Val Ala Gln Gly Gly Arg Lys Thr Thr Thr Gly Thr Arg Lys
195 200 205

Pro Arg Gly Leu Glu Pro Arg Arg Arg Lys Val Lys Thr Thr Val Val
210 215 220

Tyr Gly Arg Arg Arg Ser Lys Ser Arg Gly Arg Arg Ala Pro Thr Pro
225 230 235 240

Gln Arg Ala Gly Ser Pro Leu Pro Arg Ser Ser Ser Ser His His Arg
245 250 255

Ser Phe Gly Gly Ile Leu Ala Gly Leu Ile Gly Leu Leu Val Ser Phe
260 265 270

```
Phe Leu Leu Ile Lys Ile Leu Glu Ile Leu Arg Arg Leu Asp Trp Trp
        275                 280                 285

Trp Ile Ser Leu Ser Ser Pro Lys Gly Lys Met Gln Cys Ala Phe Gln
    290                 295                 300

Asp Thr Gly Ala Gln Ile Ser Pro His Tyr Val Gly Ser Cys Pro Trp
305                 310                 315                 320

Gly Cys Pro Gly Phe Leu Trp Thr Tyr Leu Arg Leu Phe Ile Ile Phe
                325                 330                 335

Leu Leu Ile Leu Leu Val Ala Ala Gly Leu Leu Tyr Leu Thr Asp Asn
            340                 345                 350

Gly Ser Thr Ile Leu Gly Lys Leu Gln Trp Ala Ser Val Ser Ala Leu
        355                 360                 365

Phe Ser Ser Ile Ser Ser Leu Leu Pro Ser Asp Pro Lys Ser Leu Val
    370                 375                 380

Ala Leu Thr Phe Gly Leu Ser Leu Ile Trp Met Thr Ser Ser Ser Ala
385                 390                 395                 400

Thr Gln Thr Leu Val Thr Leu Thr Gln Leu Ala Thr Leu Ser Ala Leu
                405                 410                 415

Phe Tyr Lys Ser
            420
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 552 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
ATG GAC ATC GAC CCT TAT AAA GAA TTT GGA GCT ACT GTC GAG TTA CTC        48
Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu
  1               5                  10                  15

TCG TTT TTG CCT TCT GAC TTC TTT CCT TCA GTA CGA GAT CTT CTA GAT        96
Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp
             20                  25                  30

ACC GCC TCA GCT CTG TAT CGG GAA GCC TTA GAG TCT CCT GAG CAT TGT       144
Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
         35                  40                  45

TCA CCT CAC CAT ACT GCA CTC AGG CAA GCA ATT CTT TGC TGG GGG GAA       192
Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
     50                  55                  60

CTA ATG ACT CTA GCT ACC TGG GTG GGT GTT AAT TTG GAA GAT CCA GCG       240
Leu Met Thr Leu Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala
 65                  70                  75                  80
```

TCT AGA GAC CTA GTA GTC AGT TAT GTC AAC ACT AAT ATG GGC CTA AAG 288
Ser Arg Asp Leu Val Val Ser Tyr Val Asn Thr Asn Met Gly Leu Lys
85 90 95

TTC AGG CAA CTC TTG TGG TTT CAC ATT TCT TGT CTC ACT TTT GGA ACA 336
Phe Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Thr
100 105 110

GAA ACA GTT ATA GAG TAT TTG GTG TCT TTC GGA GTG TGG ATT CGC ACT 384
Glu Thr Val Ile Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
115 120 125

CCT CCA GCT TAT AGA CCA CCA AAT GCC CCT ATC CTA TCA ACA CTT CCG 432
Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
130 135 140

GAG ACT ACT GTT GTT AGA CGA CCA GGC AGG TCC CCT AGA AGA AGA ACT 480
Glu Thr Thr Val Val Arg Arg Pro Gly Arg Ser Pro Arg Arg Arg Thr
145 150 155 160

CCC TCG CCT CGC AGA CGA AGG TCT CAA TCG CCC CGT CGC AGA AGA TCT 528
Pro Ser Pro Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser
165 170 175

CAA TCT CGG GAA TCT CAA TGT TAG 552
Gln Ser Arg Glu Ser Gln Cys
180

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu
1 5 10 15

Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp
20 25 30

Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
35 40 45

Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
50 55 60

Leu Met Thr Leu Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala
65 70 75 80

Ser Arg Asp Leu Val Val Ser Tyr Val Asn Thr Asn Met Gly Leu Lys
85 90 95

Phe Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Thr
100 105 110

Glu Thr Val Ile Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
115 120 125

```
Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
    130                 135                 140

Glu Thr Thr Val Val Arg Arg Pro Gly Arg Ser Pro Arg Arg Arg Thr
145                 150                 155                 160

Pro Ser Pro Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser
                165                 170                 175

Gln Ser Arg Glu Ser Gln Cys
            180
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 681 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
ATG GAG AAC ATC ACA TCA GGA TTC CTA GGA CCC CTT CTC GTG TTA CAG      48
Met Glu Asn Ile Thr Ser Gly Phe Leu Gly Pro Leu Leu Val Leu Gln
  1               5                  10                  15

GCG GGG TTT TTC TTG TTG ACA AGA ATC CTC ACA ATA CCC CAG AGT CTA      96
Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu
             20                  25                  30

GAC TCG TGG TGG ACT TCT CTC AAT TTT CTA GGG GGA ACT ACC GTG TGT     144
Asp Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys
         35                  40                  45

CTT GGC CAA AAT TCG CAG TCC CCA ACC TCC AAT CAC TCA CCA ACC TCT     192
Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser
     50                  55                  60

TGT CCT CCA ACT TGT CCT GGT TAT CGC TGG ATG TGT CTG CGG CGT TTT     240
Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe
 65                  70                  75                  80

ATC ATC TTC CTC TTC ATC CTG CTG CTA TGC CTC ATC TTC TTG TTG GTT     288
Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val
                 85                  90                  95

CTT CTG GAC TAT CAA GGT ATG TTG CCC GTT TGT CCT CTA ATT CCA GGA     336
Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly
            100                 105                 110

TCC TCA ACA ACC AGC ACG GGA CCA TGC CGG ACC TGC ATG ACT ACT GCT     384
Ser Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala
        115                 120                 125

CAA GGA ACC TCT ATG TAT CCC TCC TGT TGC TGT ACC AAA CCT TCG GAC     432
Gln Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp
    130                 135                 140

GGA AAT TGC ACC TGT ATT CCC ATC CCA TCA TCC TGG GCT TTC GGA AAA     480
Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys
145                 150                 155                 160
```

```
TTC CTA TGG GAG TGG GCC TCA GCC CCT TTC TCC TGG CTC AGT TTA CTA         528
Phe Leu Trp Glu Trp Ala Ser Ala Pro Phe Ser Trp Leu Ser Leu Leu
                165                 170                 175

GTC CCA TTT GTT CAG TGG TTC GTA GGG CTT TCC CCC ACT GTT TGG CTT         576
Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu
            180                 185                 190

TCA GTT ATA TGG ATG ATG TGG TAT TGG GGG CCA AGT CTG TAC AGC ATC         624
Ser Val Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile
        195                 200                 205

TTG AGT CCC TTT TTA CCG CTG TTA CCA ATT TTC TTT TGT CTT TGG GTA         672
Leu Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val
    210                 215                 220

TAC ATT TAA                                                             681
Tyr Ile
225
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 226 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Glu Asn Ile Thr Ser Gly Phe Leu Gly Pro Leu Leu Val Leu Gln
 1               5                  10                  15

Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu
            20                  25                  30

Asp Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys
        35                  40                  45

Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser
    50                  55                  60

Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe
65                  70                  75                  80

Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val
                85                  90                  95

Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly
            100                 105                 110

Ser Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala
        115                 120                 125

Gln Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp
    130                 135                 140

Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys
145                 150                 155                 160
```

Phe Leu Trp Glu Trp Ala Ser Ala Pro Phe Ser Trp Leu Ser Leu Leu
                165   170   175

Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu
            180   185   190

Ser Val Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile
        195   200   205

Leu Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val
    210   215   220

Tyr Ile
225

What is claimed is:

CLAIMS

1. A method of inhibiting the replication of a naturally-occurring hepadnavirus, said method comprising the steps of:

(a) introducing into the proximity of said hepadnavirus a nucleic acid that encodes a hepadnavirus mutant polypeptide, wherein said polypeptide:

(i) comprises a first amino acid sequence that is substantially identical to a region of a wild type hepadnavirus core protein of at least 70 amino acids in length, and

(ii) lacks a second amino acid sequence of said wild type hepadnavirus core protein, wherein said second sequence comprises the carboxyterminal three amino acids of said wild type hepadnavirus core protein and does not exceed 100 amino acids in length; and

(b) allowing said mutant polypeptide to be expressed from said nucleic acid, wherein said mutant polypeptide inhibits the replication of said naturally-occurring hepadnavirus.

2. The method of claim 1, wherein said polypeptide further comprises a third amino acid sequence that is substantially identical to a portion of a wild type hepadnavirus surface protein.

3. The method of claim 1 or 2, wherein said hepadnavirus is hepatitis B virus (HBV).

4. The method of claim 1 or 2, wherein said method is used to treat an infection of hepatitis B virus in a patient.

5. The method of claim 3, wherein the carboxyterminal amino acid of said first amino acid sequence corresponds to a position selected from the group consisting positions 81 to 180 of SEQ ID NO: 12, inclusive.

6. The method of claim 3, wherein said carboxyterminal amino acid of said first amino acid sequence corresponds to a position selected from the group consisting of positions 171 to 180 of SEQ ID NO: 12, inclusive.

7. The method of claim 3, wherein said carboxyterminal amino acid of said first amino acid sequence corresponds to position 171 of SEQ ID NO: 12.

8. The method of claim 3, wherein said carboxyterminal amino acid of said first amino acid sequence corresponds to position 178 of SEQ ID NO: 12.

9. The method of claim 3, wherein said second amino acid sequence comprises amino acids 172-183 of SEQ ID NO: 12.

10. The method of claim 3, wherein the aminoterminal amino acid of said third amino acid sequence corresponds to a position selected from the group consisting of positions 1 to 112 of SEQ ID NO: 14, inclusive.

11. The method of claim 3, wherein the aminoterminal amino acid of said third amino acid sequence corresponds to a position selected from the group consisting of positions 1 to 8 of SEQ ID NO: 14, inclusive.

12. The method of claim 3, wherein said aminoterminal amino acid of said third amino acid sequence corresponds to position 5 of SEQ ID NO: 14.

13. The method of claim 3, wherein said aminoterminal amino acid of said third amino acid sequence corresponds to position 8 of SEQ ID NO: 14.

14. The method of claim 3, wherein the carboxyterminal amino acid of said third amino acid sequence corresponds to a position selected from the group consisting of positions 51 to 224 of SEQ ID NO: 14, inclusive.

15. The method of claim 3, wherein the carboxyterminal amino acid of said third amino acid sequence corresponds to a position selected from the group consisting of positions 112 to 224 of SEQ ID NO: 14, inclusive.

16. The method of claim 3, wherein the carboxyterminal amino acid of said third amino acid sequence corresponds to position 51 of SEQ ID NO: 14.

17. The method of claim 3, wherein the carboxyterminal amino acid of said third amino acid sequence corresponds to position 112 of SEQ ID NO: 14.

18. The method of claim 3, wherein the carboxyterminal amino acid of said third amino acid sequence corresponds to position 224 of SEQ ID NO: 14.

19. A method of inhibiting the replication of a naturally-occurring hepadnavirus, said method comprising

introducing into the proximity of said hepadnavirus a hepadnavirus mutant polypeptide, wherein said polypeptide:

(i) comprises a first amino acid sequence that is substantially identical to a region of a wild type hepadnavirus core protein of at least 70 amino acids in length, and

(ii) lacks a second amino acid sequence of said wild type hepadnavirus core protein, wherein said second sequence comprises the carboxyterminal three amino acids of said wild type hepadnavirus core protein and does not exceed 100 amino acids in length wherein said mutant polypeptide inhibits the replication of said hepadnavirus.

20. The method of claim 19, wherein said polypeptide further comprises a third amino acid sequence that is substantially identical to a portion of a wild type hepadnavirus surface protein.

21. A method of inhibiting the replication of a naturally-occurring hepadnavirus, said method comprising the steps of:

(a) introducing into the proximity of said hepadnavirus a nucleic acid that encodes a hepadnavirus mutant polypeptide, wherein said polypeptide comprises:

(i) a first amino acid sequence that is substantially identical to a region of a wild type hepadnavirus core protein of at least 70 amino acids, and

(ii) a second amino acid sequence that is substantially identical to a portion of a wild type hepadnavirus surface protein.

(b) allowing said mutant polypeptide to be expressed from said nucleic acid, wherein said mutant polypeptide inhibits the replication of said hepadnavirus.

22. A nucleic acid encoding a mutant hepatitis B virus (HBV) polypeptide, wherein said polypeptide:

(a) comprises a first amino acid sequence that is substantially identical to a region of a wild type HBV core protein of at least 70 amino acids in length; and

(b) lacks a second amino acid sequence of said wild type HBV core protein, wherein said second sequence comprises the carboxyterminal three amino acids of said wild type HBV core protein and does not exceed nine amino acids in length.

23. The nucleic acid of claim 22, wherein the carboxyterminal amino acid of said first amino acid sequence is selected from the group consisting of the amino acids between position 174 and position 180 of SEQ ID NO: 12, inclusive.

24. A nucleic acid encoding a mutant hepadnavirus polypeptide, wherein said polypeptide:

(a) comprises a first amino acid sequence that is substantially identical to a region of a wild type hepadnavirus core protein of at least 70 amino acids in length;

(b) lacks a second amino acid sequence of said wild type hepadnavirus core protein, wherein said second sequence comprises the carboxyterminal three amino acids of said wild type hepadnavirus core protein; and

(c) comprises a third amino acid sequence that is substantially identical to a portion of a wild type hepadnavirus surface protein.

25. The nucleic acid of claim 24, wherein said second amino acid sequence does not exceed 100 amino acids in length.

26. The nucleic acid of claim 24, wherein the carboxyterminal amino acid of said first amino acid sequence corresponds to a position selected from the group consisting of positions 71 to 180 of SEQ ID NO: 12, inclusive.

27. A nucleic acid encoding a mutant hepadnavirus polypeptide, wherein said polypeptide comprises:

(a) a first amino acid sequence that is substantially identical to a region of a wild type hepadnavirus core protein of at least 70 amino acids in length; and

(b) a second amino acid sequence that is substantially identical to a portion of a wild type hepadnavirus surface protein.

28. A polypeptide encoded by the nucleic acid of any one of claims 22, 24, or 27.

29. A vector comprising the nucleic acid of any one of claims 22, 24, or 27.

30. A therapeutic composition comprising the mutant polypeptide of claim 28 in a pharmaceutically acceptable carrier.

31. A therapeutic composition comprising the vector of claim 29 in a pharmaceutically acceptable carrier.

32. The method of claim 1, wherein said hepadnavirus is selected from the group consisting of a woodchuck hepatitis virus (WHV), a hepatitis B virus (HBV), a hepatitis delta virus (HDV), a ground squirrel hepatitis B virus, and a duck hepatitis B virus (DHBV).

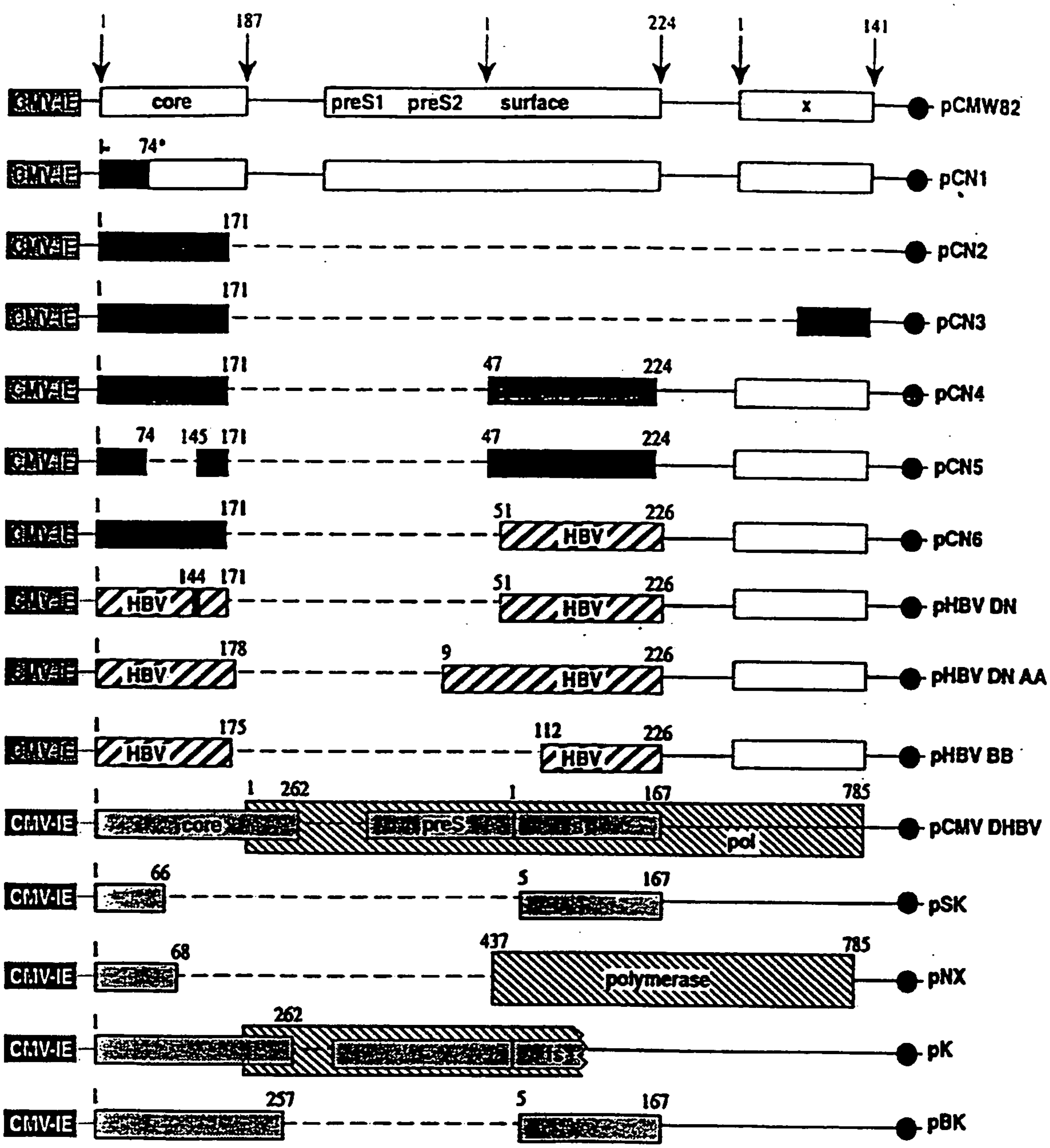

1
187
1
224
1
141
CMV-IE
core
preS1 preS2 surface
x
pCMW82
1 74*
pCN1
1 171
pCN2
1 171
pCN3
1 171 47 224
pCN4
1 74 145 171 47 224
pCN5
1 171 51 226
HBV
pCN6
1 144 171 51 226
HBV
pHBV DN
1 178 9 226
pHBV DN AA
1 175 112 226
pHBV BB
1 1 262 1 167 785
core
preS
pol
pCMV DHBV
1 66 5 167
pSK
1 68 437 785
polymerase
pNX
1 262
pK
1 257 5 167
pBK

Fig 1

Fig. 2

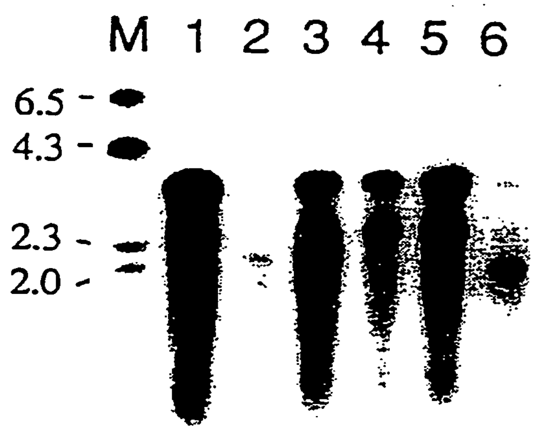


Fig. 3

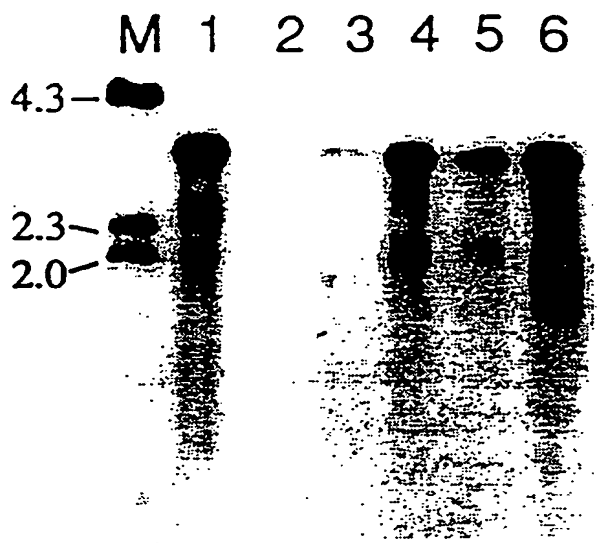


Fig. 4

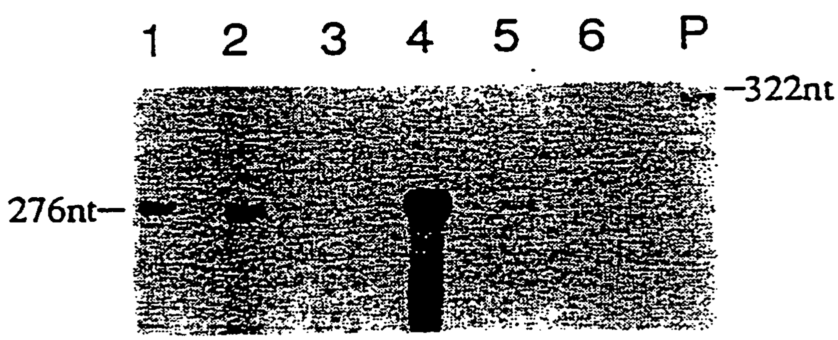
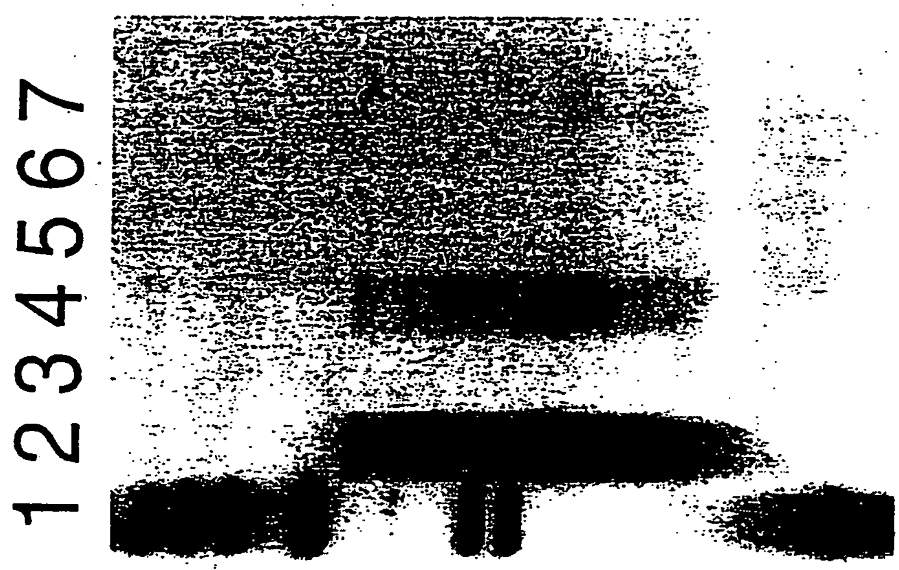


Fig. 6

Fig. 5



17:39:34

Fig. 7

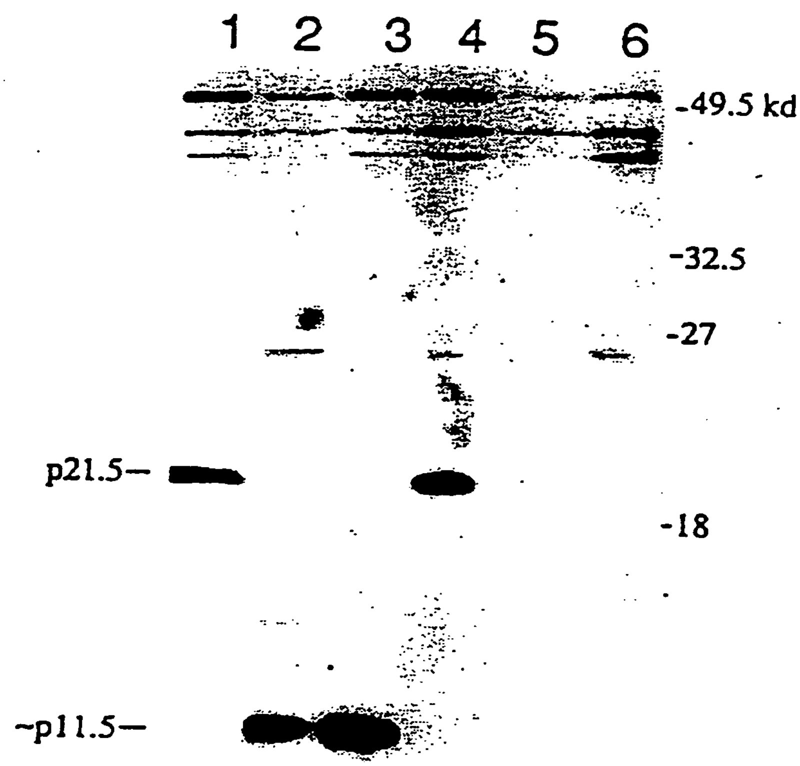


Fig. 8

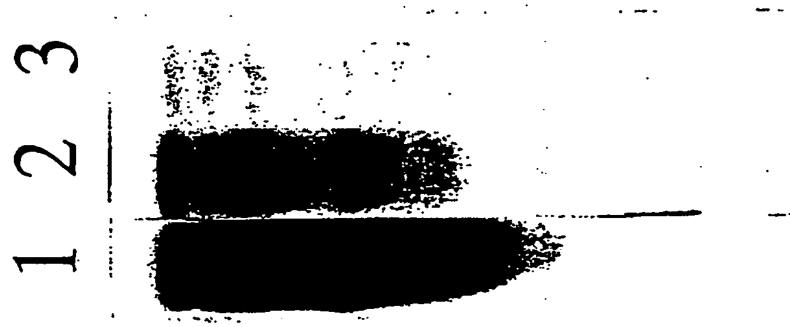


Fig. 9

1 2 3 4 5 6 7

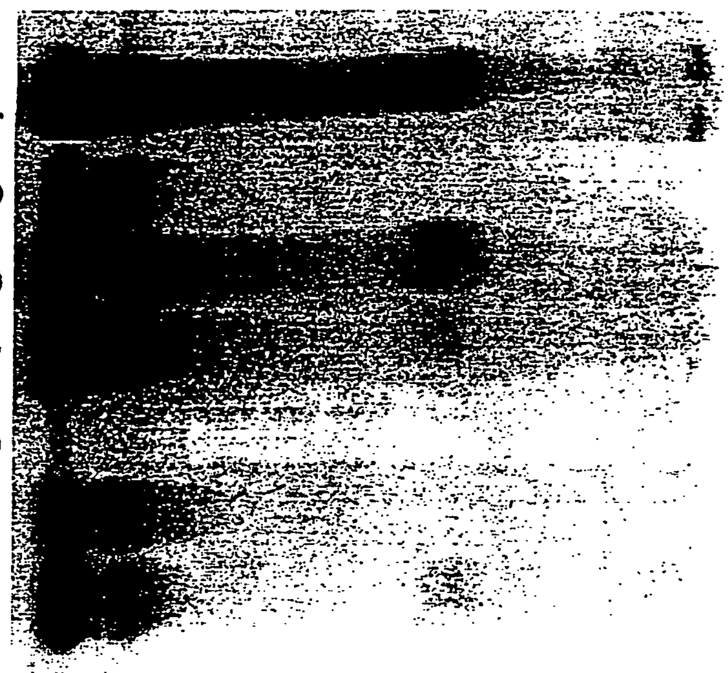


Fig. 10

```
1    /   1                                            31   /   11
ATG GAC ATA GAT CCC TAT AAA GAA TTT GGT TCA TCT TAT CAG TTG TTG AAT TTT CTT CCT
met asp ile asp pro tyr lys glu phe gly ser ser tyr gln leu leu asn phe leu pro
61   /   21                                           91   /   31
TTG GAC TTC TTT CCT GAC CTT AAT GCT TTG GTG GAC ACT GCT ACT GCC TTG TAT GAA GAA
leu asp phe phe pro asp leu asn ala leu val asp thr ala thr ala leu tyr glu glu
121  /   41                                           151  /   51
GAG CTA ACA GGT AGG GAA CAT TGC TCT CCG CAC CAT ACA GCT ATT AGA CAA GCT TTA GTA
glu leu thr gly arg glu his cys ser pro his his thr ala ile arg gln ala leu val
181  /   61                                           211  /   71
TGC TGG GAT GAA TTA ACT AAA TTG ATA GCT TGG ATG AGC TCT AAC ATA ACT TCT GAA CAA
cys trp asp glu leu thr lys leu ile ala trp met ser ser asn ile thr ser glu gln
241  /   81                                           271  /   91
GTA AGA ACA ATC ATA GTA AAT CAT GTC AAT GAT ACC TGG GGA CTT AAG GTG AGA CAA AGT
val arg thr ile ile val asn his val asn asp thr trp gly leu lys val arg gln ser
301  /  101                                           331  /  111
TTA TGG TTT CAT TTG TCA TGT CTC ACT TTC GGA CAA CAT ACA GTT CAA GAA TTT TTA GTA
leu trp phe his leu ser cys leu thr phe gly gln his thr val gln glu phe leu val
361  /  121                                           391  /  131
AGT TTT GTA GTA TGG ATC AGA ACT CCA GCT CCA TAT AGA CCT CCT AAT GCA CCC ATT CTC
ser phe val val trp ile arg thr pro ala pro tyr arg pro pro asn ala pro ile leu
421  /  141                                           451  /  151
TCG ACT CTT CCG GAA CAT ACA GTC ATT AGA AGA GGA GGT GCA AGA GCT TCT AGG TCC CCC
ser thr leu pro glu his thr val ile arg arg gly gly ala arg ala ser arg ser pro
481  /  161                                           511  /  171
AGA AGA CGC ACT CCC TCT CCT CGC AGG AGA AGA TCC CAA AAT TCG CAG TTC CAA ACT TGC
arg arg arg thr pro ser pro arg arg arg arg ser gln asn ser gln phe gln thr cys
541  /  181                                           571  /  191
AAA CAC TTG CCA ACC TCC TGT CCA CCA ACT TGC AAT GGC TTT CGT TGG ATG TAT CTG CGG
lys his leu pro thr ser cys pro pro thr cys asn gly phe arg trp met tyr leu arg
601  /  201                                           631  /  211
CGT TTT ATC ATA TAC CTA TTA GTC CTG CTG CTG TGC CTC ATC TTC TTG TTG GTT CTC CTG
arg phe ile ile tyr leu leu val leu leu leu cys leu ile phe leu leu val leu leu
661  /  221                                           691  /  231
GAC TGG AAA GGT TTA ATA CCT GTC TGT CCT CTT CAA CCC ACA ACA GAA ACA ACA GTC AAT
asp trp lys gly leu ile pro val cys pro leu gln pro thr thr glu thr thr val asn
721  /  241                                           751  /  251
TGC AGA CAA TGC ACA ATC TCT GCA CAA GAC ATG TAT ACT CCT CCT TAC TGT TGT TGT TTA
cys arg gln cys thr ile ser ala gln asp met tyr thr pro pro tyr cys cys cys leu
781  /  261                                           811  /  271
AAA CCT ACG GCA GGA AAT TGC ACT TGT TGG CCC ATC CCT TCA TCA TGG GCT TTA GGA AAT
lys pro thr ala gly asn cys thr cys trp pro ile pro ser ser trp ala leu gly asn
841  /  281                                           871  /  291
TAC CTA TGG GAG TGG GCC TTA GCT CGT CTC TCT TGG CTC AAT TTA CTA GTG CCC TTG CTT
tyr leu trp glu trp ala leu ala arg leu ser trp leu asn leu leu val pro leu leu
901  /  301                                           931  /  311
CAA TGG TTA GGA GGA ATT TCC CTC ATT GCG TGG TTT TTG CTT ATA TGG ATG ATT TGG TTT
gln trp leu gly gly ile ser leu ile ala trp phe leu leu ile trp met ile trp phe
961  /  321                                           991  /  331
TGG GGG CCC GCA CTT CTG AGC ATC TTA CCG CCA TTT ATT CCC ATA TTT GTT CTG TTT TTC
trp gly pro ala leu leu ser ile leu pro pro phe ile pro ile phe val leu phe phe
1021 /  341
TTG ATT TGG GTA TAC ATT TGA
leu ile trp val tyr ile OPA
```

Fig. 11

```
1    /   1                                    31   /   11
ATG GAC ATC GAC CCT TAT AAA GAA TTT GGA GCT ACT GTG GAG TTA CTC TCG TTT TTG CCT
met asp ile asp pro tyr lys glu phe gly ala thr val glu leu leu ser phe leu pro
61   /   21                                   91   /   31
TCT GAC TTC TTT CCT TCA GTA CGA GAT CTT CTA GAT ACC GCC TCA GCT CTG TAT CGG GAA
ser asp phe phe pro ser val arg asp leu leu asp thr ala ser ala leu tyr arg glu
121  /   41                                   151  /   51
GCC TTA GAG TCT CCT GAG CAT TGT TCA CCT CAC CAT ACT GCA CTC AGG CAA GCA ATT CTT
ala leu glu ser pro glu his cys ser pro his his thr ala leu arg gln ala ile leu
181  /   61                                   211  /   71
TGC TGG GGG GAA CTA ATG ACT CTA GCT ACC TGG GTG GGT GTT AAT TTG GAA GAT CCA GCG
cys trp gly glu leu met thr leu ala thr trp val gly val asn leu glu asp pro ala
241  /   81                                   271  /   91
TCT AGA GAC CTA GTA GTC AGT TAT GTC AAC ACT AAT ATG GGC CTA AAG TTC AGG CAA CTC
ser arg asp leu val val ser tyr val asn thr asn met gly leu lys phe arg gln leu
301  /   101                                  331  /   111
TTG TGG TTT CAC ATT TCT TGT CTC ACT TTT GGA AGA GAA ACA GTT ATA GAG TAT TTG GTG
leu trp phe his ile ser cys leu thr phe gly arg glu thr val ile glu tyr leu val
361  /   121                                  391  /   131
TCT TTC GGA GTG TGG ATT CGC ACT CCT CCA GCT TAT AGA CCA CCA AAT GCC CCT ATC CTA
ser phe gly val trp ile arg thr pro pro ala tyr arg pro pro asn ala pro ile leu
421  /   141                                  451  /   151
TCA ACA CTT CCG GAA CAT ACA GTC ATT AGA AGA GGA GGT GCA AGA GCT TCT AGG TCC CCC
ser thr leu pro glu his thr val ile arg arg gly gly ala arg ala ser arg ser pro
481  /   161                                  511  /   171
AGA AGA CGC ACT CCC TCT CCT CGC AGG AGA AGA TCC CAA AAT TCG CAG TCC CCA ACC TCC
arg arg arg thr pro ser pro arg arg arg arg ser gln asn ser gln ser pro thr ser
541  /   181                                  571  /   191 → S.
AAT CAC TCA CCA ACC TCT TGT CCT CCA ACT TGT CCT GGT TAT CGC TGG ATG TGT CTG CGG
asn his ser pro thr ser cys pro pro thr cys pro gly tyr arg trp met cys leu arg
601  /   201                                  631  /   211
CGT TTT ATC ATC TTC CTC TTC ATC CTG CTG CTA TGC CTC ATC TTC TTG TTG GTT CTT CTG
arg phe ile ile phe leu phe ile leu leu leu cys leu ile phe leu leu val leu leu
661  /   221                                  691  /   231
GAC TAT CAA GGT ATG TTG CCC GTT TGT CCT CTA ATT CCA GGA TCC TCA ACA ACC AGC ACG
asp tyr gln gly met leu pro val cys pro leu ile pro gly ser ser thr thr ser thr
721  /   241                                  751  /   251
GGA CCA TGC CGG ACC TGC ATG ACT ACT GCT CAA GGA ACC TCT ATG TAT CCC TCC TGT TGC
gly pro cys arg thr cys met thr thr ala gln gly thr ser met tyr pro ser cys cys
781  /   261                                  811  /   271
TGT ACC AAA CCT TCG GAC GGA AAT TGC ACC TGT ATT CCC ATC CCA TCA TCC TGG GCT TTC
cys thr lys pro ser asp gly asn cys thr cys ile pro ile pro ser ser trp ala phe
841  /   281                                  871  /   291
GGA AAA TTC CTA TGG GAG TGG GCC TCA GCC CGT TTC TCC TGG CTC AGT TTA CTA GTG CCA
gly lys phe leu trp glu trp ala ser ala arg phe ser trp leu ser leu leu val pro
901  /   301                                  931  /   311
TTT GTT CAG TGG TTC GTA GGG CTT TCC CCC ACT GTT TGG CTT TCA GTT ATA TGG ATG ATG
phe val gln trp phe val gly leu ser pro thr val trp leu ser val ile trp met met
961  /   321                                  991  /   331
TGG TAT TGG GGG CCA AGT CTG TAC AGC ATC TTG AGT CCC TTT TTA CCG CTC TTA CCA ATT
trp tyr trp gly pro ser leu tyr ser ile leu ser pro phe leu pro leu leu pro ile
1021 /   341
TTC TTT TGT CTT TGG GTA TAC ATT TAA
phe phe cys leu trp val tyr ile OCH
```

Fig. 12

1 / 1 31 / 11
ATG GAC ATC GAG CCT TAT AAA GAA TTT GGA GCT ACT GTG GAG TTA CTC TCG TTT TTG CCT
met asp ile asp pro tyr lys glu phe gly ala thr val glu leu leu ser phe leu pro
61 / 21 91 / 31
TCT GAC TTC TTT CCT TCA GTA CGA GAT CTT CTA GAT ACC GCC TCA GCT CTG TAT CGG GAA
ser asp phe phe pro ser val arg asp leu leu asp thr ala ser ala leu tyr arg glu
121 / 41 151 / 51
GCC TTA GAG TCT CCT GAG CAT TGT TCA CCT CAC CAT ACT GCA CTC AGG CAA GCA ATT CTT
ala leu glu ser pro glu his cys ser pro his his thr ala leu arg gln ala ile leu
181 / 61 211 / 71
TGC TGG GGG GAA CTA ATG ACT CTA GCT ACC TGG GTG GGT GTT AAT TTG GAA GAT CCA GCG
cys trp gly glu leu met thr leu ala thr trp val gly val asn leu glu asp pro ala
241 / 81 271 / 91
TCT AGA GAC CTA GTA GTC AGT TAT GTC AAC ACT AAT ATG GGC CTA AAG TTC AGG CAA CTC
ser arg asp leu val val ser tyr val asn thr asn met gly leu lys phe arg gln leu
301 / 101 331 / 111
TTG TGG TTT CAC ATT TCT TGT CTC ACT TTT GGA AGA GAA ACA GTT ATA GAG TAT TTG GTG
leu trp phe his ile ser cys leu thr phe gly arg glu thr val ile glu tyr leu val
361 / 121 391 / 131
TCT TTC GGA GTG TGG ATT CGC ACT CCT CCA GCT TAT AGA CCA CCA AAT GCC CCT ATC CTA
ser phe gly val trp ile arg thr pro pro ala tyr arg pro pro asn ala pro ile leu
421 / 141 451 / 151
TCA ACA CTT CCG GAG ACT ACT GTT GTT AGA CGA CGA GGC AGG TCC CCT AGA AGA AGA ACT
ser thr leu pro glu thr thr val val arg arg arg gly arg ser pro arg arg arg thr
481 / 161 511 / 171
CCC TCG CCT CGC AGA CGA AGG TCT CAA TCG CCG CGT CGC AGA AGA TCT CAA TCT CGG CTA
pro ser pro arg arg arg arg ser gln ser pro arg arg arg arg ser gln ser arg leu
541 / 181 571 / 191
GGA CCC CTT CTC GTG TTA CAG GCG GGG TTT TTC TTG TTG ACA AGA ATC CTC ACA ATA CCG
gly pro leu leu val leu gln ala gly phe phe leu leu thr arg ile leu thr ile pro
601 / 201 631 / 211
CAG AGT CTA GAC TCG TGG TGG ACT TCT CTC AAT TTT CTA GGG GGA ACT ACC GTG TGT CTT
gln ser leu asp ser trp trp thr ser leu asn phe leu gly gly thr thr val cys leu
661 / 221 691 / 231
GGC CAA AAT TCG CAG TCC CCA ACC TCC AAT CAC TCA CCA ACC TCT TGT CCT CCA ACT TGT
gly gln asn ser gln ser pro thr ser asn his ser pro thr ser cys pro pro thr cys
721 / 241 751 / 251
CCT GGT TAT CGC TGG ATG TGT CTG CGG CGT TTT ATC ATC TTC CTC TTC ATC CTG CTG CTA
pro gly tyr arg trp met cys leu arg arg phe ile ile phe leu phe ile leu leu leu
781 / 261 811 / 271
TGC CTC ATC TTC TTG TTG GTT CTT CTG GAC TAT CAA GGT ATG TTG CCC GTT TGT CCT CTA
cys leu ile phe leu leu val leu leu asp tyr gln gly met leu pro val cys pro leu
841 / 281 871 / 291
ATT CCA GGA TCC TCA ACA ACC AGC ACG GGA CCA TGC CGG ACC TGC ATG ACT ACT GCT CAA
ile pro gly ser ser thr thr ser thr gly pro cys arg thr cys met thr thr ala gln
901 / 301 931 / 311
GGA ACC TCT ATG TAT CCC TCC TGT TGC TGT ACC AAA CCT TCG GAC GGA AAT TGC ACC TGT
gly thr ser met tyr pro ser cys cys cys thr lys pro ser asp gly asn cys thr cys
961 / 321 991 / 331
ATT CCC ATC CCA TCA TCC TGG GCT TTC GGA AAA TTC CTA TGG GAG TGG GCC TCA GCC CGT
ile pro ile pro ser ser trp ala phe gly lys phe leu trp glu trp ala ser ala arg
1021 / 341 1051 / 351
TTC TCC TGG CTC AGT TTA CTA GTG CCA TTT GTT CAG TGG TTC GTA GGG CTT TCC CCC ACT
phe ser trp leu ser leu leu val pro phe val gln trp phe val gly leu ser pro thr
1081 / 361 1111 / 371
GTT TGG CTT TCA GTT ATA TGG ATG ATG TGG TAT TGG GGG CCA AGT CTG TAC AGC ATC TTG
val trp leu ser val ile trp met met trp tyr trp gly pro ser leu tyr ser ile leu
1141 / 381 1171 / 391
AGT CCC TTT TTA CCG CTG TTA CCA ATT TTC TTT TGT CTT TGG GTA TAC ATT TAA
ser pro phe leu pro leu leu pro ile phe phe cys leu trp val tyr ile OCH

Fig. 13

```
1   /   1                                   31  /   11
ATG GAC ATC GAC CCT TAT AAA GAA TTT GGA GCT ACT GTG GAG TTA CTC TCG TTT TTG CCT
met asp ile asp pro tyr lys glu phe gly ala thr val glu leu leu ser phe leu pro
61  /   21                                  91  /   31
TCT GAC TTC TTT CCT TCA GTA CGA GAT CTT CTA GAT ACC GCC TCA GCT CTG TAT CGG GAA
ser asp phe phe pro ser val arg asp leu leu asp thr ala ser ala leu tyr arg glu
121 /   41                                  151 /   51
GCC TTA GAG TCT CCT GAG CAT TGT TCA CCT CAC CAT ACT GCA CTC AGG CAA GCA ATT CTT
ala leu glu ser pro glu his cys ser pro his his thr ala leu arg gln ala ile leu
181 /   61                                  211 /   71
TGC TGG GGG GAA CTA ATG ACT CTA GCT ACC TGG GTG GGT GTT AAT TTG GAA GAT CCA GCG
cys trp gly glu leu met thr leu ala thr trp val gly val asn leu glu asp pro ala
241 /   81                                  271 /   91
TCT AGA GAC CTA GTA GTC AGT TAT GTC AAC ACT AAT ATG GGC CTA AAG TTC AGG CAA CTC
ser arg asp leu val val ser tyr val asn thr asn met gly leu lys phe arg gln leu
301 /   101                                 331 /   111
TTG TGG TTT CAC ATT TCT TGT CTC ACT TTT GGA AGA GAA ACA GTT ATA GAG TAT TTG GTG
leu trp phe his ile ser cys leu thr phe gly arg glu thr val ile glu tyr leu val
361 /   121                                 391 /   131
TCT TTC GGA GTG TGG ATT CGC ACT CCT CCA GCT TAT AGA CCA CCA AAT GCC CCT ATC CTA
ser phe gly val trp ile arg thr pro pro ala tyr arg pro pro asn ala pro ile leu
421 /   141                                 451 /   151
TCA ACA CTT CCG GAG ACT ACT GTT GTT AGA CGA CGA GGC AGG TCC CCT AGA AGA AGA ACT
ser thr leu pro glu thr thr val val arg arg arg gly arg ser pro arg arg arg thr
481 /   161                                 511 /   171
CCC TCG CCT CGC AGA CGA AGG TCT CAA TCG CCG CGT CGC AGA AGA TCG ATC CTC AAC AAC
pro ser pro arg arg arg arg ser gln ser pro arg arg arg arg ser ile leu asn asn
541 /   181                                 571 /   191
CAG CAC GGG ACC ATG CCG GAC CTG CAT GAC TAC TGC TCA AGG AAC CTC TAT GTA TCC CTC
gln his gly thr met pro asp leu his asp tyr cys ser arg asn leu tyr val ser leu
601 /   201                                 631 /   211
CTG TTG CTG TAC CAA ACC TTC GGA CGG AAA TTG CAC CTG TAT TCC CAT CCC ATC ATC CTG
leu leu leu tyr gln thr phe gly arg lys leu his leu tyr ser his pro ile ile leu
661 /   221                                 691 /   231
GGC TTT CGG AAA ATT CCT ATG GGA GTG GGC CTC AGC CCG TTT CTC CTG GCT CAG TTT ACT
gly phe arg lys ile pro met gly val gly leu ser pro phe leu leu ala gln phe thr
721 /   241                                 751 /   251
AGT GCC ATT TGT TCA GTG GTT CGT AGG GCT TTC CCC CAC TGT TTG GCT TTC AGT TAT ATG
ser ala ile cys ser val val arg arg ala phe pro his cys leu ala phe ser tyr met
781 /   261                                 811 /   271
GAT GAT GTG GTA TTG GGG GCC AAG TCT GTA CAG CAT CTT GAG TCC CTT TTT ACC GCT GTT
asp asp val val leu gly ala lys ser val gln his leu glu ser leu phe thr ala val
841 /   281                                 871 /   291
ACC AAT TTT CTT TTG TCT TTG GGT ATA CAT TTA
thr asn phe leu leu ser leu gly ile his leu
```

Fig. 14

```
1    /   1                                              31   /   11
ATG GAT ATC AAT GCT TCT AGA GCC TTA GCC AAT GTG TAT GAT CTA CCA GAT GAT TTC TTT
met asp ile asn ala ser arg ala leu ala asn val tyr asp leu pro asp asp phe phe
61   /   21                                             91   /   31
CCA AAA ATA GAT GAT CTT GTT AGA GAT GCT AAA GAC GCT TTA GAG CCT TAT TGG AAA TCA
pro lys ile asp asp leu val arg asp ala lys asp ala leu glu pro tyr trp lys ser
121  /   41                                             151  /   51
GAT TCA ATA AAG AAA CAT GTT TTG ATT GCA ACT CAC TTT GTG GAT CTT ATT GAA GAC TTC
asp ser ile lys lys his val leu ile ala thr his phe val asp leu ile glu asp phe
181  /   61                                             211  /   71
TGG CAG ACT ACA CAG GGC ATG CAT GAA ATA GCC GAA TCA TTA AGA GCT GTT ATA CCT CCC
trp gln thr thr gln gly met his glu ile ala glu ser leu arg ala val ile pro pro
241  /   81                                             271  /   91
ACT ACT ACT CCT GTT CCA CCG GGT TAT CTT ATT CAG CAC GAA GAA GCT GAA GAG ATA CCT
thr thr thr pro val pro pro gly tyr leu ile gln his glu glu ala glu glu ile pro
301  /   101                                            331  /   111
TTG GGA GAT TTA TTT AAA CAC CAA GAA GAA AGG ATA GTG AGT TTC CAA CCC GAC TAT CCG
leu gly asp leu phe lys his gln glu glu arg ile val ser phe gln pro asp tyr pro
361  /   121                                            391  /   131
ATT ACG GCT AGA ATT CAT GCT CAT TTG AAA GCT TAT GCA AAA ATT AAC GAG GAA TCA CTG
ile thr ala arg ile his ala his leu lys ala tyr ala lys ile asn glu glu ser leu
421  /   141                                            451  /   151
GAT AGG GCT AGG AGA TTG CTT TGG TGG CAT TAC AAC TGT TTA CTG TGG GGA GAA GCT CAA
asp arg ala arg arg leu leu trp trp his tyr asn cys leu leu trp gly glu ala gln
481  /   161                                            511  /   171
GTT ACT AAC TAT ATT TCT CGC TTG CGT ACT TGG TTG TCA ACT CCT GAG AAA TAT AGA GGT
val thr asn tyr ile ser arg leu arg thr trp leu ser thr pro glu lys tyr arg gly
541  /   181                                            571  /   191
AGA GAT GCC CCG ACC ATT GAA GCA ATC ACT AGA CCA ATC CAG GTG GCT CAG GGA GGC CGA
arg asp ala pro thr ile glu ala ile thr arg pro ile gln val ala gln gly gly arg
601  /   201                                            631  /   211
AAA ACA ACT ACG GGT ACT AGA AAA CCT CGT GGA CTC GAA CCT AGA AGA AGA AAA GTT AAA
lys thr thr thr gly thr arg lys pro arg gly leu glu pro arg arg arg lys val lys
661  /   221                                            691  /   231
ACC ACA GTT GTC TAT GGG AGA AGA CGT TCA AAG TCC CGG GGA AGG AGA GCC CCT ACA CCC
thr thr val val tyr gly arg arg arg ser lys ser arg gly arg arg ala pro thr pro
721  /   241                                            751  /   251
CAA CGT GCG GGC TCC CCT CTC CCA CGT AGT TCG AGC AGC CAC CAT AGA TCC TTC GGG GGA
gln arg ala gly ser pro leu pro arg ser ser ser ser his his arg ser phe gly gly
781  /   261                                            811  /   271
ATA CTA GCT GGC CTA ATC GGA TTA CTG GTA AGC TTT TTC TTG TTG ATA AAA ATT CTA GAA
ile leu ala gly leu ile gly leu leu val ser phe phe leu leu ile lys ile leu glu
841  /   281                                            871  /   291
ATA CTG AGG AGG CTA GAT TGG TGG TGG ATT TCT CTC AGT TCT CCA AAG GGA AAA ATG CAA
ile leu arg arg leu asp trp trp trp ile ser leu ser ser pro lys gly lys met gln
901  /   301                                            931  /   311
TGC GCT TTC CAA GAT ACT GGA GCC CAA ATC TCT CCA CAT TAC GTC GGA TCT TGC CCG TGG
cys ala phe gln asp thr gly ala gln ile ser pro his tyr val gly ser cys pro trp
961  /   321                                            991  /   331
GGA TGC CCA GGA TTT CTT TGG ACC TAT CTC AGG CTT TTT ATC ATC TTC CTC TTA ATC CTG
gly cys pro gly phe leu trp thr tyr leu arg leu phe ile ile phe leu leu ile leu
1021 /   341                                            1051 /   351
CTA GTA GCA GCA GGC TTG CTG TAT CTG ACG GAC AAC GGG TCT ACT ATT TTA GGA AAG CTC
leu val ala ala gly leu leu tyr leu thr asp asn gly ser thr ile leu gly lys leu
1081 /   361                                            1111 /   371
CAA TGG GCG TCG GTC TCA GCC CTT TTC TCC TCC ATC TCT TCA CTA CTG CCC TCG GAT CCG
gln trp ala ser val ser ala leu phe ser ser ile ser ser leu leu pro ser asp pro
1141 /   381                                            1171 /   391
AAA TCT CTC GTC GCT TTA ACG TTT GGA CTT TCA CTT ATA TGG ATG ACT TCC TCC TCT GCC
lys ser leu val ala leu thr phe gly leu ser leu ile trp met thr ser ser ser ala
1201 /   401                                            1231 /   411
ACC CAA ACG CTC GTC ACC TTA ACG CAA TTA GCC ACG CTG TCT GCT CTT TTT TAC AAG AGC
thr gln thr leu val thr leu thr gln leu ala thr leu ser ala leu phe tyr lys ser
1261 /   421
TAG
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Fig. 15

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1    /   1                                   31    /   11
ATG GAC ATC GAC CCT TAT AAA GAA TTT GGA GCT ACT GTC GAG TTA CTC TCG TTT TTG CCT
met asp ile asp pro tyr lys glu phe gly ala thr val glu leu leu ser phe leu pro
61   /   21                                  91    /   31
TCT GAC TTC TTT CCT TCA GTA CGA GAT CTT CTA GAT ACC GCC TCA GCT CTG TAT CGG GAA
ser asp phe phe pro ser val arg asp leu leu asp thr ala ser ala leu tyr arg glu
121  /   41                                  151   /   51
GCC TTA GAG TCT CCT GAG CAT TGT TCA CCT CAC CAT ACT GCA CTC AGG CAA GCA ATT CTT
ala leu glu ser pro glu his cys ser pro his his thr ala leu arg gln ala ile leu
181  /   61                                  211   /   71
TGC TGG GGG GAA CTA ATG ACT CTA GCT ACC TGG GTG GGT GTT AAT TTG GAA GAT CCA GCG
cys trp gly glu leu met thr leu ala thr trp val gly val asn leu glu asp pro ala
241  /   81                                  271   /   91
TCT AGA GAC CTA GTA GTC AGT TAT GTC AAC ACT AAT ATG GGC CTA AAG TTC AGG CAA CTC
ser arg asp leu val val ser tyr val asn thr asn met gly leu lys phe arg gln leu
301  /  101                                  331   /  111
TTG TGG TTT CAC ATT TCT TGT CTC ACT TTT GGA AGA GAA ACA GTT ATA GAG TAT TTG GTG
leu trp phe his ile ser cys leu thr phe gly arg glu thr val ile glu tyr leu val
361  /  121                                  391   /  131
TCT TTC GGA GTG TGG ATT CGC ACT CCT CCA GCT TAT AGA CCA CCA AAT GCC CCT ATC CTA
ser phe gly val trp ile arg thr pro pro ala tyr arg pro pro asn ala pro ile leu
421  /  141                                  451   /  151
TCA ACA CTT CCG GAG ACT ACT GTT GTT AGA CGA CGA GGC AGG TCC CCT AGA AGA AGA ACT
ser thr leu pro glu thr thr val val arg arg arg gly arg ser pro arg arg arg thr
481  /  161                                  511   /  171
CCC TCG CCT CGC AGA CGA AGG TCT CAA TCG CCC CGT CGC AGA AGA TCT CAA TCT CGG GAA
pro ser pro arg arg arg arg ser gln ser pro arg arg arg arg ser glu ser arg glu
541  /  181
TCT CAA TGT TAG
ser gln cys AMB
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Fig. 16

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1   /   1                                        31  /  11
ATG GAG AAC ATC ACA TCA GGA TTC CTA GGA CCC CTT CTC GTG TTA CAG GCG GGG TTT TTC
met glu asn ile thr ser gly phe leu gly pro leu leu val leu gln ala gly phe phe
61  /  21                                        91  /  31
TTG TTC ACA AGA ATC CTC ACA ATA CCC CAG AGT CTA GAC TCG TGG TGG ACT TCT CTC AAT
leu leu thr arg ile leu thr ile pro gln ser leu asp ser trp trp thr ser leu asn
121 /  41                                        151 /  51
TTT CTA GGG GGA ACT ACC GTG TGT CTT GGC CAA AAT TCG CAG TCC CCA ACC TCC AAT CAC
phe leu gly gly thr thr val cys leu gly gln asn ser gln ser pro thr ser asn his
181 /  61                                        211 /  71
TCA CCA ACC TCT TGT CCT CCA ACT TGT CCT GGT TAT CGC TGG ATG TGT CTG CGG CGT TTT
ser pro thr ser cys pro pro thr cys pro gly tyr arg trp met cys leu arg arg phe
241 /  81                                        271 /  91
ATC ATC TTC CTC TTC ATC CTG CTG CTA TGC CTC ATC TTC TTG TTG GTT CTT CTG GAC TAT
ile ile phe leu phe ile leu leu leu cys leu ile phe leu leu val leu leu asp tyr
301 / 101                                        331 / 111
CAA GGT ATG TTG CCC GTT TGT CCT CTA ATT CCA GGA TCC TCA ACA ACC AGC ACG GGA CCA
gln gly met leu pro val cys pro leu ile pro gly ser ser thr thr ser thr gly pro
361 / 121                                        391 / 131
TGC CGG ACC TGC ATG ACT ACT GCT CAA GGA ACC TCT ATG TAT CCC TCC TGT TGC TGT ACC
cys arg thr cys met thr thr ala gln gly thr ser met tyr pro ser cys cys cys thr
421 / 141                                        451 / 151
AAA CCT TCG GAC GGA AAT TGC ACC TGT ATT CCC ATC CCA TCA TCC TGG GCT TTC GGA AAA
lys pro ser asp gly asn cys thr cys ile pro ile pro ser ser trp ala phe gly lys
481 / 161                                        511 / 171
TTC CTA TGG GAG TGG GCC TCA GCC CGT TTC TCC TGG CTC AGT TTA CTA GTG CCA TTT GTT
phe leu trp glu trp ala ser ala arg phe ser trp leu ser leu leu val pro phe val
541 / 181                                        571 / 191
CAG TGG TTC GTA GGG CTT TCC CCC ACT GTT TGG CTT TCA GTT ATA TGG ATG ATG TGG TAT
gln trp phe val gly leu ser pro thr val trp leu ser val ile trp met met trp tyr
601 / 201                                        631 / 211
TGG GGG CCA AGT CTG TAC AGC ATC TTG AGT CCC TTT TTA CCG CTG TTA CCA ATT TTC TTT
trp gly pro ser leu tyr ser ile leu ser pro phe leu pro leu leu pro ile phe phe
661 / 221
TGT CTT TGG GTA TAC ATT TAA
cys leu trp val tyr ile OCH
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10602

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 48/00; C12N 5/00, 15/00
US CL :435/172.3, 240.2, 320.1; 424/93.21; 514/44; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/172.3, 240.2, 320.1; 424/93.21; 514/44; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS MEDLINE BIOSIS CAPLUS WPIDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Virology, Volume 65, Number 04, issued April 1991, BLUM ET AL., "Naturally Occurring Missense Mutation in the Polymerase Gene Terminating Hepatitis B Virus Replication", pages 1836-1842, see entire document.	1-32
Y	Virology, Volume 199, issued 1994, MELEGARI ET AL., "Properties of Hepatitis B Virus Pre-S1 Deletion Mutants", pages 292-300, see entire document.	1-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 SEPTEMBER 1996	30 SEP 1996

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